EXHIBIT 2

Applicants: David Stern et al.
U.S. Serial No.: 10/049,893
Filed: July 22, 2002

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C12N 15/12, C07K 14/705, G01N 33/566, C07K 16/28, C12N 15/10, C07K 1/22

A1

(11) International Publication Number: WO 97/39121

(43) International Publication Date: 23 October 1997 (23.10.97)

(21) International Application Number: PCT/EP97/01832

(22) International Filing Date: 11 April 1997 (11.04.97)

(30) Priority Data:
08/633.147 16 April 1996 (16.04.96) US

(71) Applicant: SCHERING AKTIENGESELLSCHAFT [DE/DE]; Patente, Müllerstrasse 178, Postfach 65 03 11, D-13342 Berlin (DE).

(72) Inventors: MORSER, Michael, John; 746 Sanchez Street, San Francisco, CA 94114 (US). NAGASHIMA, Mariko; 2409 Palmer Avenue, Belmont, CA 94002 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: ADVANCED GLYCOSYLATION END-PRODUCT RECEPTOR PEPTIDES AND USES THEREFOR

(57) Abstract

It is a general object of the present invention to provide compositions that specifically interact with advanced glycosylation end products (AGEs) or their receptors. Such compositions may be used in a variety of applications including therapeutic applications, e.g., as blocking agents to inhibit or otherwise reduce the AGE/RAGE interaction, screening applications, e.g., as models of the AGE/RAGE interaction, and diagnostic applications, e.g., to identify abnormal levels of AGE or RAGE in a given system.

Applicants: David Stern et al.
Serial No.: 10/049,893
Filed: July 22, 2002
Exhibit 2

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania
AM	Armenia
AT	Austria
AU	Australia
AZ	Azerbaijan
BA	Bosnia and Herzegovina
BB	Barbados
BE	Belgium
BF	Burkina Faso
BG	Bulgaria
BJ	Benin
BR	Brazil
BY	Belarus
CA	Canada
CF	Central African Republic
CG	Congo
CH	Switzerland
CI	Côte d'Ivoire
CM	Cameroon
CN	China
CU	Cuba
CZ	Czech Republic
DE	Germany
DK	Denmark
EE	Estonia
ES	Spain
FI	Finland
FR	France
GA	Gabon
GB	United Kingdom
GE	Georgia
GH	Ghana
GN	Guinea
GR	Greece
HU	Hungary
IE	Ireland
IL	Israel
IS	Iceland
IT	Italy
JP	Japan
KE	Kenya
KG	Kyrgyzstan
KP	Democratic People's Republic of Korea
KR	Republic of Korea
KZ	Kazakstan
LC	Saint Lucia
LI	Liechtenstein
LK	Sri Lanka
LR	Liberia
LS	Lesotho
LT	Lithuania
LU	Luxembourg
LV	Latvia
MC	Monaco
MD	Republic of Moldova
MG	Madagascar
MK	The former Yugoslav Republic of Macedonia
ML	Mali
MN	Mongolia
MR	Mauritania
MW	Malawi
MX	Mexico
NE	Niger
NL	Netherlands
NO	Norway
NZ	New Zealand
PL	Poland
PT	Portugal
RO	Romania
RU	Russian Federation
SD	Sudan
SE	Sweden
SG	Singapore
SI	Slovenia
SK	Slovakia
SN	Senegal
SZ	Swaziland
TD	Chad
TG	Togo
TJ	Tajikistan
TM	Turkmenistan
TR	Turkey
TT	Trinidad and Tobago
UA	Ukraine
UG	Uganda
US	United States of America
UZ	Uzbekistan
VN	Viet Nam
YU	Yugoslavia
ZW	Zimbabwe

ADVANCED GLYCOSYLATION END-PRODUCT RECEPTOR PEPTIDES AND USES THEREFOR

BACKGROUND OF THE INVENTION

Advanced glycosylation end-products of proteins (AGEs) have been implicated in a variety of disorders including complications associated with diabetes and normal aging. AGEs are nonenzymatically glycosylated proteins which have been shown to accumulate in vascular tissue in aging and at an accelerated rate in individuals with diabetes. Particularly, AGEs result from the non-enzymatic, but concentration dependant interaction of glucose and other reducing sugars with amino groups on proteins to form glycosylated proteins termed Amadori adducts. Over time, these Amadori adducts undergo additional rearrangements, dehydrations and cross-linking with other proteins to accumulate as a family of complex structures referred to as AGEs.

AGEs have been shown to bind specifically in a saturable and reversible manner to cell surface receptors, including receptors expressed on the surface of endothelial cells and particularly those of the microvasculature, monocytes/macrophages, smooth muscle cells, mesengial cells and neurons.

Following binding to cell surface receptors, AGEs are taken up in vesicles and either degraded intracellularly or transported through the cells and deposited in the sub-endothelial matrix, where they accumulate. Esposito et al., J. Exp. Med. 170:1387-1407 (1989). In addition, chemotactic signals for monocytes, but not other white cells are released. These monocytes then adhere and diapedese through the endothelial cell layer. Kirstein et al., Proc. Nat'l Acad. Sci. USA 187:9010-9014 (1990).

AGEs also have been shown to cause proliferation of endothelial cells, which become more permeable and more thrombogenic, i.e., thrombomodulin is downregulated while tissue factor is upregulated. Esposito et al., J. Exp. Med. 170:1387-1407 (1989).

Monocytes\macrophages can also take up AGEs through their receptors which are distinct from the acetyl-LDL receptors, but which may be related to the receptors for aldehyde-modified proteins. Takata et al., J. Biol. Chem. 263:14819-14825 (1988), Takata et al., Biochim. Biophys. Acta 986:18-26 (1989), Radoff et al., Diabetes 39:1510-1518 (1990). Binding of AGEs to monocytes in vitro leads to the induction of cytokines, TNF and IL-1, which then stimulate the release of a number of other growth factors responsible for cell proliferation, migration and matrix synthesis. Vlassara et al., Science 240:1546-1548 (1988). Increased

synthesis of matrix proteins in response to AGEs binding has also been demonstrated for mesangial cells. Skolnick et al., J. Exp. Med. 174:931-939 (1991); Doi et al., Proc. Nat'l Acad. Sci. USA 89:2873-2877 (1992).

Evidence has indicated that the binding of AGEs to their receptors either directly or indirectly induces inflammatory responses in vessel walls, which can trigger or aggravate the pathogenesis of diabetic macro- or microangiopathy. For an overview of the effects of AGEs, see, e.g., Vlassara et al., Laboratory Invest. 70(2):138-151 (1994). Stern et al., J. Biol. Chem. 267:14998-15004 (1992) has reported similarities between RAGE proteins and the Ig superfamily molecules.

Because of the effects AGEs may have in the pathogenesis of a number of disorders, it would generally be desirable to provide compositions and methods to block or otherwise inhibit these effects, and particularly the interaction between AGEs and their cell surface receptors. The present invention meets these and a variety of other needs.

SUMMARY OF THE INVENTION

It is a general object of the present invention to provide novel polypeptides and antibodies, as well as methods of using these polypeptides and antibodies in screening, diagnostic and therapeutic applications.

In a first aspect, the present invention provides substantially pure polypeptides which comprise a biologically active, soluble, human RAGE polypeptide. The present invention also provides pharmaceutical compositions which present the polypeptides of the invention with a pharmaceutically acceptable carrier.

The polypeptides of the invention may be used in a number of applications. For example, in a further aspect, the polypeptides may be used in methods for purifying ligands to RAGE, e.g., AGEs. The methods generally comprise the steps of immobilizing the soluble human RAGE polypeptide on a solid support, followed by contacting the immobilized RAGE polypeptide with a mixture of proteins containing an AGE whereby the RAGE polypeptide selectively binds the AGE in the mixture of proteins. The immobilized RAGE polypeptide is then washed to remove unbound proteins and the purified AGE which was selectively bound to the immobilized RAGE is eluted.

In another aspect, the invention provides models of AGE/AGE receptor interactions to identify effectors of this interaction. For example, the invention provides a method of screening a test compound to determine if that compound is an agonist or antagonist of a AGE/AGE receptor interaction. The method comprises separately incubating a soluble human RAGE polypeptide with

an AGE in the presence and absence of the test compound. The level of interaction between the soluble human RAGE polypeptide and the AGE in the presence and absence of the test compound is then detected. The level of interaction in the presence and absence of the test compound is then compared. An increase or decrease in the level of interaction in the presence of the test compound is indicative that the test compound is an agonist or antagonist of the interaction, respectively.

In an additional aspect, the present invention provides a method of inhibiting an interaction between an AGE and a receptor which specifically binds AGE, by contacting the AGE with an effective amount of a soluble human RAGE polypeptide.

In a related aspect, the present invention provides methods of treating patients for symptoms of a disorder which are caused by an interaction between an AGE and its receptor. The method comprises administering an effective amount of a soluble human RAGE polypeptide to the patient.

In still another aspect, the present invention provides nucleic acid sequences which encode soluble human RAGE polypeptides.

Further, the present invention provides the use of an effective amount of a soluble human RAGE polypeptide for the production of a pharmaceutical compound or composition for the treatment of a patient for symtoms of a disorder, wherein said symptoms are caused by an interaction between an AGE and its receptor. Especially, said patients are diabetic patients, and said symptom is increased vascular permeability. Especially, said disorder is Diabetes Mellitus and said symptom is selected from the group consisting of diabetic microvasculopathy, diabetic macrovasculopathy and occlusive vascular disorder.
Especially, said symptoms are selected from neuropathy, nephropathy, retinopathy and atherosclerosis.
Especially, said symptom is hemodialysis-associated amyloidosis.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A and 1B show the nucleic acid sequence and deduced amino acid sequence of a soluble human RAGE polypeptide (Seq ID NOS: 1-4). Figure 1A shows the DNA and amino acid sequence of soluble human RAGE which includes an expressed pre-sequence (Seq ID NOS: 1-2), whereas Figure 1B shows the sequences for mature soluble human RAGE (Seq ID NOS: 3-4). Standard three-letter abbreviations are used to denote the individual amino acids.

Figures 2A and 2B show binding of an AGE-BSA protein to indirectly immobilized RAGE polypeptide. Figure 2A shows total binding of labeled AGE-BSA to RAGE immobilized indirectly upon a solid support through a FLAG peptide/antiFLAG antibody interaction which mimics the cell surface presentation of RAGE (squares). Also shown is nonspecific binding (diamonds)(labeled AGE-BSA binding in the presence of excess unlabeled AGE-BSA). Figure 2B shows corrected, specific binding of AGE-BSA to RAGE polypeptide that is indirectly coupled to the solid support.

Figure 3 shows a dose response curve for RAGE/AGE binding in the presence of increasing concentrations of free RAGE

Figure 4A is a bar graph showing levels of antibody binding to human soluble RAGE using an EIA antibody capture assay. Binding is compared between intact soluble RAGE (dark bars) and RAGE/DCC chimeric protein (in which the first Ig-like domain of RAGE is replaced with the first Ig-like domain of DCC, a member of the Ig superfamily. Four antibodies (#5, 9, 14 and 19) recognized both the intact soluble RAGE and the chimeric protein. Figures 4B and 4C show antibody binding to peptide fragments of soluble human RAGE, designated peptide 1 (CKGAPKKPPQ) (Seq ID NO: 5), fragment 2 (WKLNTGRTEAC) (Seq ID NO: 6) and fragment 8 (GPQDQGTYSC) (Seq ID NO: 7).

Figure 5 shows a Western Blot hybridization of anti-RAGE MAb SW1E8 (ATCC Accession No. HB-12166). Lanes 1-3 represent rat, mouse and human RAGE expressed in Baculovirus, respectively. Lanes were loaded with 5 µl of conditioned media. Monomeric RAGE is apparent as a doublet at approximately 41 Kd.

Figures 6A and 6B show results of a direct antigen capture EIA. Figure 6A shows a capture assay employing MAb RBF9D9 (ATCC Accession No. HB-12165) as the RAGE capture antibody and MAb SW10C1 (ATCC Accession No. HB-12164) as the RAGE detection antibody, which recognizes human, but not murine RAGE. Figure 6B shows a capture assay employing MAb RBF9D9 (ATCC

Accession No. HB-12165) as the RAGE capture antibody and MAb SW1E8 (ATCC Accession No. HB-12166) as the detection antibody, which recognizes both human and murine RAGE.

Figure 7 shows the results of flow cytometry of CHO parental cells and CHO cells transfected with full length human RAGE, using anti-RAGE MAb SW10C1 (ATCC Accession No. HB-12164). Sixty nine of the original 72 MAb panel demonstrated similar reactivity with cell surface RAGE.

Figures 8A and 8B show results of flow cytometry of CHO parental and CHO-RAGE transfectants incubated with lactoferrin and immunostained with anti-RAGE/phycoerythrin (Figure 8A) and anti-lactoferrin/FITC (Figure 8B).

Figures 9A and 9B show the in vitro permeability of confluent BEAC layers by albumin and inulin. Permeability is shown following incubation with medium (control, white bar), normal RBCs (black bar) and diabetic RBCs (hatched bars). Reversal of diabetic RBC associated permeability is demonstrated following pretreatment with soluble, recombinant RAGE (sRAGE) but not control protein (sVCAM).

Figure 10 shows plasma pharmacokinetics of ^{125}I-human recombinant RAGE after an intravenous infusion into rat.

Figures 11A and 11B show comparisons of efficiency of recombinant soluble human RAGE and recombinant rat-VCAM in reversing permeability induced by diabetic RBC incubation in various tissues of normal (Figure 11A) and diabetic rats (Figure 11B).

Figure 12 shows a schematic illustration of the blood-tissue albumin transport tracer uptake method. Also shown are the calculations used to obtain albumin clearance values.

Figures 13A, 13B and 13C show albumin clearance from various tissues in control rats (white bar), diabetic rats (left black bar), diabetic rats with soluble RAGE pretreatment (middle black bar) and diabetic rats with soluble VCAM-1 pretreatment (right black bar) in early (Figure 13A), mid (Figure 13B) and late (Figure 13C) phases of STZ-induced diabetes.

Figures 14A, 14B and 14C show extravascular water levels from various tissues in control rats (white bar), diabetic rats (left black bar), diabetic rats with soluble RAGE pretreatment (middle black bar) and diabetic rats with soluble VCAM-1 pretreatment (right black bar) in early (Figure 14A), mid (Figure 14B) and late (Figure 14C) phases of diabetes.

Figures 15A and 15B show adhesion of RAGE pretreated and control RBCs to single vessels.

DETAILED DESCRIPTION OF THE INVENTION

I. General

The major pathological conditions in which AGEs have been implicated as exerting adverse effects include Diabetes Mellitus in which hyperglycemia exists, as well as a number of aging related disorders. AGE formation in biological systems is dependant upon blood-glucose concentration and time of incubation. Without being bound to a particular theory, it is believed that this time/concentration dependency accounts for the adverse effects in hyperglycemic diabetics as well as the elderly. Similarly, proteins which themselves have a longer half-life, are more prone to undergo glycosylation to form AGEs. In diabetic patients, a high level of plasma glucose leads to glycosylation of various plasma proteins, including hemoglobin and LDL (low-density lipoprotein) as well as enzymes and matrix proteins. The accumulation of these AGEs induce a number of permanent abnormalities in the extracellular matrix component function, and stimulate cytokines and reactive oxygen species production through AGE-specific receptors. Inhibition of AGE formation in long term diabetic animals has also been shown to prevent or reduce the severity of a number of elements of the pathology of diabetes, including retinopathy, nephropathy, neuropathy and arterial abnormalities. Brownlee, Ann. Rev. Med. 46:223-234 (1995), Zimmerman et al., Proc. Nat'l Acad. Sci. USA 92:3744-3748 (1995).

A number of proteins associated with Alzheimer's disease, e.g., amyloid, tau, the major components of neurofibrillary tangles and senile plaques, are found to be similarly modified. Smith et al., Nature 374:316 (1995), Smith et al.,Proc. Nat'l Acad. Sci. USA, 91:5710-5714 (1994), Vitek et al., Proc. Nat'l Acad. Sci. USA, 91:4766-4770 (1994). Additionally, in hemodialysis-associated amyloidosis, β_2-microglobulin, ~~a major component of amyloid fibrils,~~ is modified by glycosylation. Miyata et al., J. Clin. Invest. 93:521-528 (1994). In atherosclerosis, in both the diabetic and non-diabetic populations, LDL, Lp(a)(lipoprotein little a) and immunoglobulin that are trapped and/or cross-linked to the matrix within the vessel wall are modified by glycosylation. The presence of AGEs produces a local, chronic inflammation, through a number of mechanisms including receptor-mediated pathway and oxidant stress. As a result, it is apparent that AGE modification causes and/or aggravates these various pathological conditions.

Because of these effects of AGEs in the pathogenesis of various disorders, it is a general object of the present invention to provide compositions that specifically interact with advanced glycosylation end products (AGEs) or their receptors. Such compositions may be used in a variety of applications including

therapeutic applications, e.g., as blocking agents to inhibit or otherwise reduce the AGE/RAGE interaction, screening applications, e.g., as models of the AGE/RAGE interaction, and diagnostic applications, e.g., to identify abnormal levels of AGE or RAGE in a given system.

In preferred aspects, the present invention provides compositions comprising soluble RAGE polypeptides, antibodies that are specifically immunoreactive with soluble RAGE polypeptides, and methods of using these compositions in screening, therapeutic and diagnostic applications.

II. Polypeptides

In a first aspect, the present invention provides substantially pure or isolated polypeptides that are related to and/or derived from human RAGE polypeptides. The terms "substantially pure" or "isolated", when referring to proteins and polypeptides, denote those polypeptides that are separated from proteins or other contaminants with which they are naturally associated. A protein or polypeptide is considered substantially pure when that protein makes up greater than about 50% of the total protein content of the composition containing that protein, and typically, greater than about 60% of the total protein content. More typically, a substantially pure or isolated protein or polypeptide will make up from about 75 to about 90% of the total protein. Preferably, the protein will make up greater than about 90%, and more preferably, greater than about 95% of the total protein in the composition.

In particular aspects, the isolated polypeptides of the present invention are related to and/or derived from soluble human RAGE polypeptides. As used herein, the term "soluble" generally refers to RAGE derived polypeptides that lack a transmembrane region that is associated with full length RAGE polypeptides. Thus, soluble RAGE polypeptides generally comprise fragments of the extracellular domain of RAGE. In certain embodiments, the soluble peptides of the invention will comprise one or more of the Ig-like domains of the extracellular region of RAGE.

As used herein, "AGE" refers to an advanced glycosylation end-product. Typically, such AGEs may be full length proteins, polypeptides or aggregations of proteins and/or polypeptides.

The polypeptides of the invention also may be characterized by their ability to either mimic or inhibit the interaction between AGEs and their receptors, e.g., RAGE. Those polypeptides which are mimetic of either AGE or its receptors in the AGE/receptor interaction are termed AGE or AGE receptor "mimics".

In particularly preferred aspects, the polypeptides of the invention will have an amino acid sequence that is related to or derived from the amino acid

sequence of soluble human RAGE as shown in Figures 1A and 1B (Seq ID NOS: 1-4). Although described in terms of the amino acid sequence shown in Figure 1A and 1B (Seq ID NOS: 1-4), it will be readily understood that the polypeptides of the present invention include those peptides having the listed amino acid sequence or biologically active fragments thereof, as well as those polypeptides having amino acid sequences that are substantially homologous to the listed sequence.

The terms "substantially homologous" when referring to polypeptides, refer comparatively to two amino acid sequences which, when optimally aligned, are at least about 75% homologous, preferably at least about 85% homologous more preferably at least about 90% homologous, and still more preferably at least about 95% homologous. Optimal alignment of sequences for aligning a comparison window may be conducted by the local homology algorithm of Smith and Waterman (1981) Adv. Appl. Math. 2:482, by the homology alignment algorithm of Needleman and Wunsch (1970) J. Mol. Biol. 48:443, by the search for similarity method of Pearson and Lipman (1988) Proc. Natl. Acad. Sci. (USA) 85:2444, or by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package Release 7.0, Genetics Computer Group, 575 Science Dr., Madison, WI).

The term "biologically active fragment" as used herein, refers to portions of the soluble RAGE polypeptides, which possess a particular biological activity. For example, such biological activity may include the ability to bind a particular protein, substrate or ligand, e.g., AGE, to have antibodies generated against it, to block or otherwise inhibit an interaction between two proteins, e.g., a receptor and its ligand, such as AGE and RAGE, between an enzyme and its substrate, between an epitope and an antibody, or such fragments may include a particular catalytic activity. With regard to the polypeptides of the present invention, particularly preferred polypeptides or biologically active fragments include, e.g., polypeptides that possess one or more of the biological activities described above, such as the ability to specifically interact with AGEs, the ability to block, reduce, or otherwise inhibit the interaction between AGEs and RAGE, and the ability to elicit antibodies that are specifically immunoreactive with AGEs or RAGE. Those fragments that are specifically recognized and bound by antibodies raised against the polypeptides of the invention are also included in the definition of biologically active fragments. Such fragments are also referred to herein as "immunologically active fragments." Examples of immunologically active fragments include those fragments comprising the amino acid sequences specifically described below, and particularly those selected from the group consisting of: WKLNTGRTEA (Seq ID NO: 8), CEVPAQPSPQI (Seq ID NO: 9), CRAMNQNGKETKSN (Seq ID NO: 10),

GPQDQGTYSC (Seq ID NO: 11), AQNITARIGEPLVLK (Seq ID NO: 12), CKGAPKKPPQ (Seq ID NO: 13), EQTRRHPET (Seq ID NO: 14), RGGDPRPTFSC (Seq ID NO: 15), SPGLPRHRAL (Seq ID NO: 16), and SSHGPQESRA (Seq ID NO: 17).

As described previously, the polypeptides of the invention may further include modifications to the N- or C-termini, i.e., acetylation, amidation, or inclusion of additional amino acids, i.e., cysteine, to assist in conjugation with other proteins or compounds, e.g., polypeptides having the following sequences: WKLNTGRTEAC (Seq ID NO: 6); AQNITARIGEPLVLKC (Seq ID NO: 18); CEQTRRHPET (Seq ID NO: 19); CSPGLPRHRAL (Seq ID NO: 20); and SSHGPQESRAC (Seq ID NO: 21).

The polypeptides of the invention may also be characterized by their ability to block the interaction between two proteins, e.g. AGE and RAGE, RAGE and anti-RAGE Abs, or AGE and anti-AGE Abs. In particular, included in the polypeptides of the present invention are peptides derived from human RAGE proteins that are capable of blocking or otherwise inhibiting the interaction between human RAGE and its ligands and particularly, AGEs. Examples of such polypeptides include fragments of human RAGE which encompass the AGE binding regions of the RAGE protein as well as AGE-binding proteins that sterically interfere with RAGE/AGE binding.

As referenced above, the polypeptides of the present invention may also be characterized by their ability to bind antibodies raised against proteins or polypeptides having the amino acid sequences of soluble human RAGE, as shown in Figure 1A and 1B (Seq ID NOS: 1-4) , or fragments thereof. These antibodies generally recognize polypeptides that are homologous to at least portions of human RAGE proteins or their immunologically active fragments. A variety of immunoassay formats may be used to select antibodies specifically immunoreactive with a particular protein or domain. For example, solid-phase ELISA immunoassays are routinely used to select monoclonal antibodies specifically immunoreactive with a protein. See Harlow and Lane (1988) Antibodies, A Laboratory Manual, Cold Spring Harbor Publications, New York, for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity. Antibodies to the polypeptides of the present invention are discussed in greater detail, below.

Generally, the biologically active fragments of the polypeptides described herein may include any subsequence of the above described RAGE polypeptide. Typically, however, such biologically active fragments will range in size from about 10 amino acids in length to about 320 amino acids in length. More typically, the biologically active fragments will be from about 10, 11, 12, 13, 14, 15 or 16 amino acids in length to about 50 amino acids in length.

Examples of particularly preferred biologically active fragments of soluble human RAGE include peptides comprising an amino acid sequence selected from the group consisting of WKLNTGRTEA (Seq ID NO: 8), CEVPAQPSPQI (Seq ID NO: 9), CRAMNQNGKETKSN (Seq ID NO: 10), GPQDQGTYSC (Seq ID NO: 11), AQNITARIGEPLVLK (Seq ID NO: 12), CKGAPKKPPQ (Seq ID NO: 13), EQTRRHPET (Seq ID NO: 14), RGGDPRPTFSC (Seq ID NO: 15), SPGLPRHRAL (Seq ID NO: 16), and SSHGPQESRA (Seq ID NO: 17).

The polypeptides of the present invention may generally be prepared using recombinant or synthetic methods that are well known in the art. Recombinant techniques are generally described in Sambrook, et al., Molecular Cloning: A Laboratory Manual, (2nd ed.) Vols. 1-3, Cold Spring Harbor Laboratory, (1989). The term recombinant is also intended to encompass the recombinant expression of heterologous genetic material which is introduced into a host cell or transfectant through viral transfection techniques. Techniques for the synthesis of polypeptides are generally described in Merrifield, J. Amer. Chem. Soc. 85:2149-2456 (1963), Atherton, et al., Solid Phase Peptide Synthesis: A Practical Approach, IRL Press (1989), and Merrifield, Science 232:341-347 (1986). In preferred aspects, the polypeptides of the present invention may be expressed by a suitable host cell that has been transfected with a nucleic acid of the invention, as described in greater detail below.

Biologically active fragments of the above described polypeptides may generally be identified and prepared using methods well known in the art. For example, selective proteolytic digestion, recombinant deletional methods or *de novo* peptide synthesis methods may be employed to identify portions of the above described peptides that possess the desired biological activity, e.g., AGE binding, presence of immunological determinants, and the like. See, e.g., Sambrook, et al.

Isolation and purification of the polypeptides of the present invention can be carried out by methods that are generally well known in the art. For example, the polypeptides may be purified using readily available chromatographic methods, *e.g.*, ion exchange, hydrophobic interaction, HPLC or affinity chromatography, to achieve the desired purity. Affinity chromatography may be particularly attractive in allowing an individual to take advantage of the specific biological activity of the desired peptide, e.g., AGE binding, presence of antigenic determinants or the like. For example, antibodies raised against human RAGE polypeptides or its immunologically active fragments, may be coupled to a suitable solid support and contacted with a mixture of proteins containing the polypeptides of the invention under conditions conducive to the association of these polypeptides

with the antibody. Once bound to the immobilized antibody, the solid support is washed to remove unbound material and/or nonspecifically bound proteins. The desired polypeptides may then be eluted from the solid support in substantially pure form by, e.g., a change in salt, pH or buffer concentration. Alternatively, the affinity of the soluble RAGE polypeptides for AGEs may be used advantageously to purify these peptides. In particular, AGEs, e.g. BSA-AGE, may be immobilized as described above, for use as affinity probes in the purification of the soluble RAGE polypeptides.

In addition to those polypeptides and fragments described above, the present invention also provides fusion proteins which contain these polypeptides or fragments. Fusion proteins may be useful in providing for enhanced expression of the RAGE polypeptide constructs, or in producing RAGE polypeptides having other desirable properties, e.g., labeling groups, e.g., enzymatic reporter groups, binding groups, antibody epitopes, etc.

The term "fusion protein" as used herein, generally refers to a composite protein, i.e., a single contiguous amino acid sequence, made up of two distinct, heterologous polypeptides which are not normally fused together in a single amino acid sequence. Thus, a fusion protein may include a single amino acid sequence that contains two entirely distinct amino acid sequences or two similar or identical polypeptide sequences, provided that these sequences are not normally found together in a single amino acid sequence. Fusion proteins may generally be prepared using either recombinant nucleic acid methods, i.e., as a result of transcription and translation of a gene fusion, which fusion comprises a segment encoding a polypeptide of the invention and a segment encoding a heterologous protein, or by chemical synthesis methods well known in the art.

Also included within the present invention are amino acid variants of the above described polypeptides. These variants may include insertions, deletions and substitutions with other amino acids. For example, in some aspects, conservative amino acid substitutions may be made, i.e., substitution of selected amino acids with different amino acids having similar structural characteristics, e.g., net charge, hydrophobicity and the like. Examples of such conservative substitutions include, e.g., Ala:Val:Leu:Ile:Met, Asp:Glu, Lys:Arg, Asn:Gln, Phe:Tyr and Ser:Thr Glycosylation modifications, either changed, increased amounts or decreased amounts, as well as other sequence modifications are also included within the polypeptides of the invention.

Systematic substitution of one or more amino acids of a consensus sequence with a D-amino acid of the same type (*e.g.*, D-lysine in place of L-lysine) may also be used to generate more stable peptides. In addition, constrained

peptides comprising a consensus sequence or a substantially identical consensus sequence variation may be generated by methods known in the art (Rizo and Gierasch (1992) Ann. Rev. Biochem. 61:387; for example, by adding internal cysteine residues capable of forming intramolecular disulfide bridges which cyclize the peptide. Similarly, modification of the amino or carboxy terminals may also be used to confer stabilizing properties upon the polypeptides of the invention, e.g., amidation of the carboxy-terminus or acylation of the amino-terminus. Substitution of amino acids involved in catalytic activity can be used to generate dominant negative inhibitors of signaling pathways.

Furthermore, although primarily described in terms of "proteins" or "polypeptides" one of skill in the art, upon reading the instant specification, will appreciate that these terms also include structural analogs and derivatives of the above-described polypeptides, e.g., polypeptides having conservative amino acid insertions, deletions or substitutions, peptidomimetics, and the like. For example, in addition to the above described polypeptides which consist only of naturally-occurring amino acids, peptidomimetics of the polypeptides of the present invention are also provided. Peptide analogs are commonly used in the pharmaceutical industry as non-peptide drugs with properties analogous to those of the template peptide. These types of non-peptide compounds are termed "peptide mimetics" or "peptidomimetics" (Fauchere, J. (1986) Adv. Drug Res. 15:29; Veber and Freidinger (1985) TINS p.392; and Evans et al. (1987) J. Med. Chem. 30:1229, and are usually developed with the aid of computerized molecular modeling. Peptide mimetics that are structurally similar to therapeutically useful peptides may be used to produce an equivalent therapeutic effect. Generally, peptidomimetics are structurally similar to a paradigm polypeptide (*i.e.*, a polypeptide that has a biological or pharmacological activity), such as naturally-occurring receptor polypeptides, but have one or more peptide linkages optionally replaced by a linkage selected from the group consisting of: $-CH_2NH-$, $-CH_2S-$, $-CH_2-CH_2-$, $-CH=CH-$ (cis and trans), $-COCH_2-$, $-CH(OH)CH_2-$, and $-CH_2SO-$, by methods known in the art and further described in the following references: Spatola, A.F. in Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins, B. Weinstein, eds., Marcel Dekker, New York, p. 267 (1983); Spatola, A.F., Vega Data (March 1983), Vol. 1, Issue 3, "Peptide Backbone Modifications" (general review); Morley, J.S., Trends Pharm. Sci. (1980) pp. 463-468 (general review); Hudson, D. et al., Int. J. Pept. Prot. Res. (1979) 14:177-185 ($-CH_2NH-$, $-CH_2CH_2-$); Spatola, A.F. et al., Life Sci (1986) 38:1243-1249 ($-CH_2-S-$); Hann, M.M., J. Chem. Soc. Perkin Trans. I (1982) 307-314 ($-CH=CH-$, cis and trans); Almquist, R.G. et al., J. Med. Chem. (1980) 23:1392-1398 ($-COCH_2-$); Jennings-White, C. et al., Tetrahedron Lett. (1982)

23:2533 (-$COCH_2$-); Szelke, M. et al., European Appln. EP 45665 (1982) CA: 97:39405 (1982) (-$CH(OH)CH_2$-); Holladay, M.W. et al., Tetrahedron Lett. (1983) 24:4401-4404 (-$C(OH)CH_2$-); and Hruby, V.J., Life Sci. (1982) 31:189-199 (-CH_2-S-).

Peptide mimetics may have significant advantages over polypeptide embodiments, including, for example: more economical production; greater chemical stability; enhanced pharmacological properties (half-life, absorption, potency, efficacy, etc.); altered specificity (*e.g.*, a broad-spectrum of biological activities); reduced antigenicity; and others.

For many applications, it will also be desirable to provide the polypeptides of the invention as labeled entities, i.e., covalently attached or linked to a detectable group, to facilitate identification, detection and quantification of the polypeptide in a given circumstance. These detectable groups may comprise a detectable protein group, e.g., an assayable enzyme or antibody epitope as described above in the discussion of fusion proteins. Alternatively, the detectable group may be selected from a variety of other detectable groups or labels, such as radiolabels (e.g., ^{125}I, ^{32}P or ^{35}S) or a chemiluminescent or fluorescent group. Similarly, the detectable group may be a substrate, cofactor, inhibitor or affinity ligand. Labeling of peptidomimetics usually involves covalent attachment of one or more labels, directly or through a spacer (*e.g.*, an amide group), to non-interfering position(s) on the peptidomimetic that are predicted by quantitative structure-activity data and/or molecular modeling. Such non-interfering positions generally are positions that do not form direct contacts with the molecules to which the peptidomimetic binds (*e.g.*, AGE) to produce the therapeutic effect. Derivitization (*e.g.*, labeling) of peptidomimetics should not substantially interfere with the desired biological or pharmacological activity of the peptidomimetic. Generally, peptidomimetics of peptides of the invention bind to their ligands (*e.g.*, AGEs) with high affinity and/or possess detectable biological activity (*i.e.*, are agonistic or antagonistic to AGE/RAGE interaction and phenotypic changes brought about by those interactions).

III. Antibodies to RAGE

In an additional aspect, the present invention provides antibodies that are specifically immunoreactive with human RAGE and more particularly, the soluble human RAGE polypeptides of the invention. The phrase "specifically immunoreactive," when referring to the interaction between an antibody of the invention and a particular protein, refers to an antibody that specifically recognizes and binds with relatively high affinity to the protein of interest, e.g., RAGE, such that this binding is determinative of the presence of the protein in a heterogeneous

population of proteins and other biologics. Thus, under designated immunoassay conditions, the specified antibodies bind to a particular protein and do not bind in a significant amount to other proteins present in the sample. A variety of immunoassay formats may be used to select antibodies specifically immunoreactive with a particular protein. For example, solid-phase ELISA immunoassays are routinely used to select monoclonal antibodies specifically immunoreactive with a protein. See, Harlow and Lane (1988) Antibodies, A Laboratory Manual, Cold Spring Harbor Publications, New York, for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity.

For production of polyclonal antibodies, an appropriate target immune system is selected, typically a mouse or rabbit, but also including goats, sheep, cows, guinea pigs, monkeys and rats. The substantially purified antigen is presented to the immune system in a fashion determined by methods appropriate for the animal. These and other parameters are well known to immunologists. Typically, injections are given in the footpads, intramuscularly, intradermally or intraperitoneally. The immunoglobulins produced by the host can be precipitated, isolated and purified by routine methods, including affinity purification.

For monoclonal antibodies, appropriate animals will be selected and the desired immunization protocol followed. After the appropriate period of time, the spleens of these animals are excised and individual spleen cells are fused, typically, to immortalized myeloma cells under appropriate selection conditions. Thereafter, the cells are clonally separated and the supernatants of each clone are tested for the production of an appropriate antibody specific for the desired region of the antigen. Techniques for producing antibodies are well known in the art. *See, e.g.*, Goding et al., Monoclonal Antibodies: Principles and Practice (2d ed.) Acad. Press, N.Y., and Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, New York (1988). Other suitable techniques involve the *in vitro* exposure of lymphocytes to the antigenic polypeptides or alternatively, to selection of libraries of antibodies in phage or similar vectors. Huse et al., Generation of Large Combinatorial Library of the Immunoglobulin Repertoire in Phage Lambda, Science 246:1275-1281 (1989). Monoclonal antibodies with affinities of 10^8 liters/mole, preferably 10^9 to 10^{10} or stronger, will be produced by these methods.

The antibodies generated can be used for a number of purposes, *e.g.*, as probes in immunoassays, for inhibiting interaction between AGEs and their receptors, in diagnostic or therapeutic applications. These applications are discussed in greater detail, below. Where the antibodies are used to block the interaction between AGEs and their receptors, the antibody will generally be referred to as a "blocking antibody."

The antibodies of the present invention can be used with or without modification. Frequently, the antibodies will be labeled by joining, either covalently or non-covalently, a substance which provides for a detectable signal. Such labels include those that are well known in the art, such as the labels described previously for the polypeptides of the invention, e.g., radioactive, fluorescent or bioactive labels. As labeled binding entities, the antibodies of the invention may be particularly useful in, e.g., diagnostic applications, for identifying abnormal levels of RAGE in human tissue or blood samples which abnormal levels may be indicative of the existence of or enhanced potential for those disorders associated with excessive RAGE/AGE interaction, as described herein.

In alternative aspects, the antibodies of the present invention may be used as affinity ligands in the quantitation and/or purification of the RAGE polypeptides from a mixture of proteins. These antibody affinity purification methods are well known in the art, and typically involve the immobilization of a particular antibody, e.g., an antibody to soluble human RAGE, upon a solid support. Solid supports for use in affinity chromatography are generally commercially available from, e.g., Sigma Chemical Co. (St Louis MO) and Pharmacia (Uppsala, Sweden).

Additionally, the antibodies of the invention may be chimeric, human-like or humanized, in order to reduce their potential antigenicity, without reducing their affinity for their target. Chimeric, human-like and humanized antibodies have generally been described in the art. Generally, such chimeric, human-like or humanized antibodies comprise hypervariable regions, *e.g.*, complementarity determining regions (CDRs) from a mammalian animal, *i.e.*, a mouse, and a human framework region. See, e.g., Queen, et al., Proc. Nat'l Acad. Sci. USA 86:10029 (1989), Verhoeyan, et al., Science 239:1534-1536 (1988). By incorporating as little foreign sequence as possible in the hybrid antibody, the antigenicity is reduced. Preparation of these hybrid antibodies may be carried out by methods well known in the art.

Preferred antibodies are those monoclonal or polyclonal antibodies which specifically recognize and bind to human RAGE proteins and more particularly, those that specifically bind to the human soluble RAGE polypeptides of the invention. Accordingly, these preferred antibodies will specifically recognize and bind the polypeptides which have an amino acid sequence that is substantially homologous to the amino acid sequence shown in Figure 1A and 1B (Seq ID NOS: 1-4) , or immunologically active fragments thereof. Still more preferred are antibodies which are capable of forming an antibody-ligand complex with the

polypeptides of the invention, whereby the ability of the RAGE polypeptides to associate with their ligands, in vitro, is reduced, e.g., blocking antibodies.

Also preferred are blocking antibodies which inhibit or reduce binding of RAGE to other natural and pathology associated ligands of human RAGE, e.g., amphoterin, β-amyloid peptides, and the like.

IV. Nucleic Acids and Cell Lines

In another aspect, the present invention provides nucleic acids which encode the polypeptides of the invention, as well as expression vectors that include these nucleic acids, and cell lines and organisms that are capable of expressing these nucleic acids. These nucleic acids, expression vectors and cell lines may generally be used to produce the polypeptides of the invention. Generally, the isolated nucleic acids of the present invention encode a polypeptide which is derived from or related to a soluble human RAGE polypeptide or biologically active fragment thereof.

In preferred aspects, the nucleic acid compositions of the invention will typically include a coding region which encodes a polypeptide having an amino acid sequence that is substantially homologous to the amino acid sequence shown in Figure 1A and 1B (Seq ID NOS: 1-4). Preferred nucleic acids will typically encode polypeptides having an amino acid sequence which is substantially homologous to the amino acid sequence shown in Figure 1A and 1B (Seq ID NOS: 1-4), or biologically active fragments thereof. Such fragments will generally comprise a segment of from about 15 to about 150 nucleotides. These fragments can be useful as oligonucleotide probes in the methods of the present invention, or alternatively to encode the polypeptides or biologically active fragments of the present invention, described herein. Also provided are substantially similar nucleic acid sequences, allelic variations and natural or induced sequences of the above described nucleic acids. Also included are chemically modified and substituted nucleic acids, *e.g.*, those which incorporate modified nucleotide bases or which incorporate a labelling group.

More preferred nucleic acids will comprise a segment having more than about 20 contiguous nucleotides from the nucleotide sequences shown in either of Figure 1A or 1B (Seq ID NOS: 1-4), with still more preferred nucleic acids having a nucleotide sequence that is substantially homologous to either of the nucleotide sequences shown in Figure 1A or 1B (Seq ID NOS: 1-4). Most preferred nucleic acids are those which include a portion, i.e., at least 20 contiguous nucleotides, or all of the nucleotide sequence shown in Figures 1A or B (Seq ID NOS: 1-4).

"Nucleic acids" of the present invention include RNA, cDNA, genomic DNA, synthetic forms and mixed polymers, both sense and antisense strands. Furthermore, different alleles of each isoform are also included. The present invention also provides recombinant nucleic acids which are not otherwise naturally occurring. The nucleic acids described herein also include self replicating plasmids and infectious polymers of DNA or RNA. Unless specified otherwise, conventional

notation for nucleic acids is used herein. For example, as written, the left hand end of a single stranded polynucleotide sequence is the 5'-end, whereas the right-hand end is the 3'-end. The left hand direction of double-stranded polynucleotide sequences is referred to as the 5' direction. The direction of 5' to 3' addition of nascent RNA transcripts is referred to as the transcription direction; sequence regions on the DNA strand having the same sequence as the RNA and which are 5' to the 5' end of the RNA transcript are referred to as "upstream sequences"; sequence regions on the DNA strand having the same sequence as the RNA and which are 3' to the 3' end of the RNA transcript are referred to as "downstream sequences".

The phrase "nucleic acid sequence encoding" refers to a nucleic acid which directs the expression of a specific protein or peptide. The nucleic acid sequences include both the DNA strand sequence that is transcribed into RNA and the RNA sequence that is translated into protein. The nucleic acid sequences include both the full length nucleic acid sequences as well as non-full length sequences derived from the full length protein. It will be further understood that the nucleic acids of the invention also encompass degenerate codons of the native sequence or sequences which may be introduced to provide codon preference in a specific host cell.

Substantial homology in the nucleic acid context means that the segments, or their complementary strands, when compared, are the same when properly aligned, with the appropriate nucleotide insertions or deletions, in at least about 60% of the nucleotides, typically, at least about 70%, more typically, at least about 80%, usually, at least about 90%, and more usually, at least about 95% to 98% of the nucleotides. Alternatively, substantial homology exists when the segments will hybridize under selective hybridization conditions to a strand, or its complement, typically using a sequence of at least about 20 contiguous nucleotides derived from the nucleotide sequences shown in Figures 1A or 1B (Seq ID NOS: 1-4) . However, larger segments will usually be preferred, *e.g.*, at least about 30 contiguous nucleotides, more usually about 40 contiguous nucleotides, and preferably more than about 50 contiguous nucleotides. Selective hybridization exists when hybridization occurs which is more selective than total lack of specificity. *See*, Kanehisa, Nucleic Acid Res. 12:203-213 (1984). Examples of such selective hybridization conditions include, e.g., hybridization under the hybridization and wash conditions of 50% formamide at 42°C. Other stringent hybridization conditions may also be selected. Generally, stringent conditions are selected to be about 5° C lower than the thermal melting point (Tm) for the specific sequence at a defined ionic strength and pH. The Tm is the temperature (under

defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Typically, stringent conditions will be those in which the salt concentration is at least about 0.02 molar at pH 7 and the temperature is at least about 60°C. As other factors may significantly affect the stringency of hybridization, including, among others, base composition and size of the complementary strands, the presence of organic solvents and the extent of base mismatching, the combination of parameters is more important than the absolute measure of any one.

The nucleic acids of the present invention may be present in whole cells, cell lysates or in partially pure or substantially pure or isolated form. When referring to nucleic acids, the terms "substantially pure" or "isolated" generally refer to the nucleic acid separated from contaminants with which it is generally associated, *e.g.*, lipids, proteins and other nucleic acids. The substantially pure or isolated nucleic acids of the present invention will be greater than about 50% pure. Typically, these nucleic acids will be more than about 60% pure, more typically, from about 75% to about 90% pure and preferably from about 95% to about 98% pure.

There are various methods of isolating the nucleic acids which encode the polypeptides of the present invention. Typically, the DNA is isolated from a genomic or cDNA library using labeled oligonucleotide probes specific for sequences in the desired DNA. Restriction endonuclease digestion of genomic DNA or cDNA containing the appropriate genes can be used to isolate the DNA encoding the polypeptides of the invention. From the nucleotide sequence given in Figures 1A or 1B (Seq ID NOS: 1-4) , a panel of restriction endonucleases can be constructed to give cleavage of the DNA in desired regions, *i.e.*, to obtain segments which encode biologically active polypeptides or fragments of the invention. Following restriction endonuclease digestion, DNA encoding the polypeptides of the invention is identified by its ability to hybridize with a nucleic acid probe in, for example, a Southern blot format. These regions are then isolated using standard methods. *See, e.g.*, Sambrook, et al., *supra*.

The polymerase chain reaction, or "PCR" can also be used to prepare nucleic acids which encode the polypeptides of the present invention. PCR technology is used to amplify nucleic acid sequences of the desired nucleic acid, *e.g.*, the DNA which encodes the polypeptides of the invention, directly from mRNA, cDNA, or genomic or cDNA libraries. Alternatively, solid phase oligonucleotide synthesis methods may also be employed to produce the nucleic acids described herein. Such methods include the phosphoramidite method described by, e.g., Beaucage and Carruthers, Tetrahedron Lett. 22:1859-1862 (1981), or the triester

method according to Matteucci, *et al.*, J. Am. Chem. Soc., 103:3185 (1981). A double stranded fragment may then be obtained, if desired, by annealing the chemically synthesized single strands together under appropriate conditions or by synthesizing the complementary strand using DNA polymerase with an appropriate primer sequence.

Appropriate primers and probes for amplifying the nucleic acids described herein, may be generated from analysis of the nucleic acid sequences described herein, e.g., in Figure 1A or 1B (Seq ID NOS: 1-4) . Briefly, oligonucleotide primers complementary to the two 3' borders of the DNA region to be amplified are synthesized. The PCR is then carried out using the two primers. *See, e.g.*, PCR Protocols: A Guide to Methods and Applications (Innis, M., Gelfand, D., Sninsky, J. and White, T., eds.) Academic Press (1990). Primers can be selected to amplify a variety of different sized segments from the nucleic acid sequence.

In addition to their use in producing the polypeptides of the invention, the nucleic acid sequences described herein are also particularly useful in a number of other applications. For example, in a particular aspect, the nucleic acid sequences of the present invention or fragments thereof, may be readily employed as nucleic acid probes useful in obtaining genes which encode the polypeptides of the present invention or other closely related genes. "Nucleic acid probes" may be DNA or RNA fragments. DNA fragments can be prepared, for example, by digesting plasmid DNA, or by use of PCR, or synthesized by either the phosphoramidite or phosphotriester methods described in, e.g., Gait, Oligonucleotide Synthesis: A Practical Approach, IRL Press (1990). Where a specific sequence for a nucleic acid probe is given, it is understood that the complementary strand is also identified and included. The complementary strand will work equally well in situations where the target is a double-stranded nucleic acid. Typical nucleic acid probes may be readily derived from the nucleotide sequence shown in Figure 1A or B (Seq ID NOS: 1-4), or alternatively, may be prepared from the amino acid sequence of soluble human RAGE polypeptides, as shown in Figure 1A or 1B (Seq ID NOS: 1-4). In particular, probes may be prepared based upon segments of the amino acid sequence which possess relatively low levels of degeneracy, *i.e.*, few or one possible nucleic acid sequences which encode therefor. Suitable synthetic DNA fragments may then be prepared.

Such nucleic acid probes, e.g., cDNA probes, may be used in the design of oligonucleotide probes and primers for screening and cloning genes which encode the polypeptides of the invention or related polypeptides, *e.g.*, using well known PCR techniques. These nucleic acids, or fragments may comprise part

Vectors and Insect Cell Culture Procedures, Texas Agricultural Station, College Station, Texas (1987).

V. Methods of Use

The compositions of the present invention have a wide variety of uses, including, inter alia, screening, diagnostic and therapeutic applications.

A. Screening Applications

In a preferred aspect, the polypeptides of the invention may be used as model systems for identifying effectors of the AGE/RAGE interaction. In particular, these model systems may be used to screen collections or libraries of test compounds in order to identify agonists or antagonists of AGE/RAGE interaction. Generally, agonists, antagonists or test compounds may be chemical compounds, mixtures of chemical compounds, biological macromolecules, or extracts made from biological materials such as bacteria, plants, fungi, or animal cells or tissues. Particularly targeted test compounds will typically include the polypeptides or fragments of the present invention as well as structural analogs or peptidomimetics which are derived from these polypeptides or the antibodies described herein, substrates or ligands thereof. As used herein, the term "agonist" refers to a composition or compound that will enhance the particular observed activity, e.g., AGE/RAGE binding, while an "antagonist" will diminish the particular observed activity. The terms "agonist" and "antagonist", as used herein, do not imply any particular mechanism of function.

The screening methods of the present invention typically involve the incubation of a polypeptide of the present invention, e.g., a soluble human RAGE polypeptide, in the presence of a standard advanced glycosylation end-product protein (AGE) such as AGE-BSA, nonenzymatically N-glycosylated collagen, myelin or the like, as well as the test compound. Typically, one of the RAGE polypeptide or AGE will be immobilized upon a solid support which will then be contacted with the other protein or peptide. The other, non-immobilized member of the AGE/RAGE pair will typically include a labeling group covalently or otherwise attached so as not to interfere with the AGE/RAGE interaction. Labeling groups will generally include those that are substantially set out above. Immobilization of one of the AGE or RAGE polypeptide permits ready separation of AGE/RAGE complex, which will be bound to the solid support, from unreacted or free AGE or RAGE, utilizing a simple wash step.

A number of suitable solid supports may be employed for immobilization of the AGE or RAGE polypeptides. Examples of suitable solid

B. Affinity Ligands

In addition to their use as screening systems, the peptides of the invention may also be used as affinity ligands which specifically bind to AGEs. As affinity ligands, these polypeptides may also be useful in the purification of AGEs from a mixture of different proteins. Affinity purification of AGEs may be carried out using affinity purification methods that well known in the art. For example, the soluble RAGE polypeptide or peptides may be attached to a suitable solid support as described above. Many solid supports are commercially available from, *e.g.*, Sigma Chemical Co., St Louis, Missouri, or Pharmacia, Uppsala, Sweden, and come prepared for immediate coupling of affinity ligands.

The mixture of proteins may be contacted with the polypeptide bound to the solid support, such that the RAGE polypeptide immobilized upon the solid support can selectively bind the AGEs within the mixture of proteins. The bound protein can then be washed to eliminate unbound proteins. Finally, substantially pure AGEs may be eluted from the solid support by generally known elution protocols, *e.g.*, changing buffer conditions, temperature, or level of carbohydrate in the elution buffer.

As affinity probes, the polypeptides of the invention may also be used to bind AGEs both *in vitro* and *in vivo*. This binding may be used in assay formats to label and detect AGEs in a sample, imaging formats to identify localization of AGEs in a patient, or in therapeutic applications to deliver a drug to areas which are relatively high in AGE concentration, or specifically deliver a drug, e.g., a proteolytic drug, to an AGE.

In a similar manner, the antibodies of the invention may also be used as affinity probes or ligands for soluble RAGE polypeptides. In particular, the specificity of the antibodies of the invention may be exploited in the purification and/or identification of RAGE polypeptides and particularly, soluble human RAGE polypeptides.

C. Diagnostic Applications

As alluded to above, the polypeptides of the invention may be used as probes capable of specifically interacting with their ligands, i.e., AGEs. As a result, the polypeptides of the invention may be used in a variety of diagnostic applications. For example, those polypeptides of the invention that are capable of specifically interacting with AGEs may be particularly useful in identifying patients who may suffer from abnormal levels of AGEs which are indicative of particular disorders, or may be viewed as indicators of future problems, such as diabetic vasculopathy.

In a particular aspect, soluble human RAGE polypeptides may be used as affinity probes to identify the presence, absence and/or relative quantity of AGEs in a sample, e.g., blood or tissue samples from a patient. This allows the identification of patients having elevated levels of AGE or other ligands of RAGE, which levels are indicative of a variety of pathological conditions associated with, e.g., Diabetes Mellitus, peripheral occlusive vascular diseases, hemodialysis-associated amyloidosis, Alzheimer's disease and other age-related disorders. Once a patient is diagnosed as suffering from one or more of these complications, or is identified as being at a higher risk, appropriate preventative or therapeutic measures may be taken, such as administration of appropriate pharmaceutical agents, e.g., compositions comprising the peptides, peptidomimetics or antibodies of the invention.

In a similar manner, the antibodies of the present invention, e.g., those that are specifically immunoreactive with human RAGE polypeptides, may be used to diagnose disorders characterized by abnormal levels or localization of AGE/RAGE interactions. In particular, the described antibodies may be used as diagnostic tools to evaluate plasma and tissue levels of RAGE in patients suffering from pathological conditions associated with elevated levels of AGE/RAGE interaction. In particular, the antibodies described herein may be used in well known immunoassay formats, e.g., ELISA, Western blotting, immunohistochemistry and FACS methods, to identify levels of RAGE in samples.

D. Therapeutic Applications

In addition to the above described uses, the soluble RAGE polypeptides and antibodies of the invention may also be used in therapeutic applications for the treatment of human or non-human mammalian patients. The term "treatment" as used herein, refers to the full spectrum of treatments for a given disorder from which the patient is suffering, including alleviation of one, most or all symptoms resulting from that disorder, to an outright cure for the particular disorder or prevention of the onset of the disorder.

In particular, the polypeptides and antibodies of the invention are useful in treating disorders or symptoms of which result from excessive levels of AGEs in tissue or plasma. As described previously, the association of AGEs and RAGE has been implicated as a symptom or causative event in a number of pathological conditions including, e.g., complications associated with Diabetes Mellitus, e.g., diabetic microvasculopathy (neuropathy, nephropathy and retinopathy), diabetic macrovasculopathy (atherosclerosis), occlusive vascular disorders, activation of microglial cells by β-amyloid peptides in Alzheimer's

disease, hemodialysis-associated amyloidosis and age related disorders such as oxidant stress. Accordingly, treatment or prevention of such disorders may generally be carried out by reducing, inhibiting or outright blocking the interaction between AGEs and RAGE. Blocking this interaction typically involves administering to a patient an effective amount of a soluble human RAGE polypeptide, peptidomimetc or blocking antibody, as described above.

The term "patient" generally refers to a mammalian individual, typically human, who has been diagnosed as suffering from one or more of the above described disorders, or who has been characterized as belonging to a group that has an abnormally high incidence of such disorders, e.g., diabetics and the elderly.

The term "effective amount" or "therapeutically effective amount" generally refers to the quantities of reagents necessary for effective therapy, i.e., the partial or complete alleviation of the symptom or disorder for which treatment was sought. Included within the definition of effective therapy are preventative treatments intended to reduce the likelihood of onset of the above-described symptoms or disorders. The effective amount for a given therapy, whether curative or preventative, will generally depend upon many different factors, including means of administration, target site, physiological state of the patient and other medicants administered. Thus, treatment doses will need to be titrated to optimize safety and efficacy. Typically, dosages used *in vitro* may provide useful guidance in the amounts useful for *in situ* administration of these reagents. Animal testing of effective doses for treatment of particular disorders will provide further predictive indication of human dosage. Generally, therapeutically effective amounts of the polypeptides or blocking antibodies of the present invention will be from about 0.0001 to about 10 mg/kg, and more usually, from about 0.001 to about 0.1 mg/kg of the host's body weight. Various considerations are described, *e.g.*, in Gilman et al., (Eds.), *Goodman and Gilman's: The Pharmacological Basis of Therapeutics*, (8th ed. 1990), Pergamon Press, and *Remington's Pharmaceutical Sciences* (7th ed. 1985) Mack Publishing Co., Easton, Penn.

Methods of administration, also discussed in the above references, include, *e.g.*, oral, intravenous, intraperitoneal or intramuscular administration, and local administration, including topical, transdermal diffusion and aerosol administration, for therapeutic, and/or prophylactic treatment. The active agent, *i.e.*, the polypeptide or antibody component, will generally be administered in a composition additionally comprising a pharmaceutically acceptable carrier. Suitable pharmaceutically acceptable carriers include water, saline, buffers and other compounds described in, *e.g.*, the Merck Index, Merck and Co., Rahway, New Jersey. For some methods of administration, *e.g.*, oral, it may be desirable to

provide the active ingredient in a liposomal formulation. This is particularly desirable where the active ingredient may be subject to degradative environments, for example, proteolytic digestive enzymes. Liposomal formulations are well known in the art, and are discussed in, *e.g.*, Remington's Pharmaceutical Sciences, *supra*. Administration may also be carried out by way of a controlled release composition or device, whereby a slow release of the active ingredient allows continuous administration over a longer period of time.

Constituents of pharmaceutical compositions, in addition to the active agents described herein, include those generally known in the art for the various administration methods used. For example, oral forms generally include powders, tablets, pills, capsules, lozenges and liquids. Similarly, intravenous, intraperitoneal or intramuscular formulations will generally be dissolved or suspended in a pharmaceutically acceptable carrier, *e.g.*, water, buffered water, saline and the like. Additionally, these compositions may include additional constituents which may be required to approximate physiological conditions, such as pH adjusting and buffering agents, tonicity adjusting agents, wetting agents and the like. For solid compositions, conventional nontoxic solid carriers may be used which include, *e.g.*, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium carbonate and the like.

Administration may also be carried out by way of a controlled release composition or device, whereby a slow release of the active ingredient allows continuous administration over a longer period of time.

The present invention is further illustrated by the following examples. These examples are merely to illustrate aspects of the present invention and are not intended as limitations of this invention.

VI. Examples

Example 1: Cloning, Expression and Purification of Soluble Human RAGE

A DNA fragment coding for human soluble RAGE was obtained from lung cDNA library using polymerase chain reaction techniques (PCR, GeneAmp, Perlin-Elmer Cetus); primers used were 5'-GATGGCAGCCGG AACAGCAGTT-3' (Seq ID NO: 22) and 5'-CTCAAGTTCCCAGCCCTGATCCTCC-3' (Seq ID NO: 23). The DNA sequence of the PCR product was confirmed by the dideoxy chain termination method (Sanger, et al., Proc. Nat'l Acad. Sci. USA 74:5463-5467 (1977). The DNA fragment was subcloned into the pCRII™ vector (Invitrogen, San Diego, CA) and the EcoR1 fragment of the resulting plasmid was cloned into the pBacPAK8 vector (Clontech, Palo Alto, CA) under control of the AcMNPV polyhedrin promoter (ATCC Accession Nos. VR-2538 and VF25-39). Baculovirus expression of recombinant human soluble RAGE was performed by co-transfecting the plasmid pBacPAK8/RAGE with a linearized BacPAK6 viral cDNA (Clontech) into Spodoptera frugiperda (Sf9) cells according to the manufacturer's instructions. Recombinant plaques were identified and purified by their beta-galactosidase negative phenotype.

Sf9 cells expressing soluble RAGE were grown as follows: Non-infected Sf 9 cells were grown in shake flasks at 28°C, to a density of 1 - 1.2 x 10^6/ml in TNMF (Grace's with supplements from Sigma) plus 10% FBS (e.g. Hyclone) and 0.1% pluronic F-68 (Sigma), and a viability of >97%. One liter of cells was infected with viral stock (MOI of ~ 0.01). Cultures were harvested 3 days post-infection by centrifuging the media at 1200 rpm for 8 min. This media was then used for purification of recombinant soluble RAGE.

Recombinant human soluble RAGE was purified from the Sf9 media by chromatography on an SP Sepharose fast flow column (Pharmacia) followed by a size exclusion chromatography step. In particular, a 1/10 volume of 1.0 M Tris-HCl, pH 8.0 was added to Sf 9 cell media to precipitate viral proteins and the media was allowed to sit at 4°C for several hours to allow precipitation. The precipitated media was centrifuged at ~ 3000 rpm for 10 to 15 min to remove any precipitate. The supernatant was diluted 1:4 with deionized water, adjusted to pH 7.5 and sterile filtered (0.2 μm). The filtered supernatant was loaded onto SP Sepharose fast flow column (#17-0729-01, Pharmacia), that had been equilibrated with 20 mM NaPO4, pH 7.5. The column was eluted in a salt gradient from 0 to 0.5 M NaCl, and the fractions were analyzed by SDS-PAGE. Fractions containing RAGE were pooled, concentrated and diafiltered into PBS buffer.

Further purification was obtained by applying the pooled fractions to a Superdex 200PG column, and again, fractions were analyzed by SDS-PAGE and pooled as appropriate.

Example 2: Solid Phase Binding Assays for Soluble Human RAGE

Two assay formats were used to assess the ability of soluble RAGE to bind AGE. The first provided a RAGE polypeptide immobilized directly to the solid support whereas the second method employed an indirect attachment to more closely mimic the cell surface presentation of the AGE binding portion of RAGE.

A. Preparation of AGE-BSA and ^{125}I-AGE-BSA

Bovine serum albumin (from Sigma, Cat #A7888) was incubated at a concentration of 25 mg/ml in phosphate buffered saline (calcium- and magnesium-free) with 250 mM ribose, in the presence of 1.5 mM PMSF (phenylmethylsulfonyl fluoride) and 1 mM EDTA (ethylenediaminetetraacetic acid). The pH of the solution was adjusted to between 6.8 and 7.0. The solution was sterilized by filtering through 0.22 micron filters and incubated in dark, at 37°C for 6 to 8 weeks. The solution containing AGE-BSA was dialysed against calcium- and magnesium-free PBS, and stored frozen in aliquots at -20°C.

100 μg of AGE-BSA was iodinated with 1 mCi of Na^{125}I using iodogen method according to the manufacturer's specifications. 4 μl of AGE-BSA (~ 100 μg), 8 μl of Na^{125}I (1 mCi) and 88 μl of phosphate buffered saline was added to a tube containing iodo-gen (Pierce). The tube was incubated for 15 minutes on ice, with occasional agitation. The reaction was terminated by adding 50 μl of 0.1% potassium iodide. The reaction mixture was passed over a desalting column to remove free iodine.

B. Competitive Binding of AGE to Indirectly Immobilized RAGE

A 96-well plate (Immunolon 4 from Dynatech Lab) was coated with anti-flag peptide antibodies (Eastman Kodak), 100 μl/well of 17 μl/ml in sodium bicarbonate buffer, pH 9.6) at 4°C overnight. The wells were washed and blocked as above. The wells were then incubated with Sf 9 media containing human recombinant soluble RAGE/flag fusion protein (75 μl of 1:4 dilution with PBS) 1 to

2 h at 37°C (flag peptide: DYKDDDDK). The wells were again washed as before and incubated with various concentrations of ^{125}I-AGE-BSA alone or in the presence of excess cold AGE-BSA (i.e., nonradioactive) in PBS containing 0.2% BSA (45 μl/well) at room temperature for 2-3 hours. The sample having an excess of cold AGE-BSA was used to account for nonspecific interactions. The wells were washed twice with 0.2% BSA in PBS. The bound ligand was then eluted with PBS containing 1 mg/ml of heparin and 1 mg/ml of BSA (100 μl/well) by incubating at 37°C for 5 minutes and counted. Figure 2 shows a graph of AGE binding to indirectly immobilized RAGE (expressed as CPM) as a function of increasing ligand concentration, in the absence and presence of excess nonradioactive ligand (Figure 2A), and corrected for nonspecific interactions (Figure 2B).

Example 3: Competition Binding Assays Using Soluble Human RAGE

Competition assays were also performed between ^{125}I-AGE-BSA and an excess of a number of other proteins, including soluble RAGE, Soluble RAGE/flag fusion protein, recombinant soluble RAGE/DCC chimeric protein (where the first Ig-like domain of RAGE is substituted with the first Ig-like domain of DCC, another Ig-superfamily member), lactoferrin and native BSA. The results are shown in Table 1, below.

Table 1

Results of Competition Binding Assay (% Binding)			
	Exp. 1	Exp. 2	Exp. 3
^{125}I-AGE-BSA only	100%	100%	100%
+ cold AGE-BSA	29%	14%	12%
+ rec. solRAGE	n.d.	65%	60%
+ rec. solRAGE/flag fusion	8%	n.d.	n.d.
+ rec. solRAGE/DCC chimeric	40%	n.d.	n.d.
+ lactoferrin	n.d.	50%	20%
+ native BSA	n.d.	>100%	>100%

n.d.- Not determined

Binding assays were done in quadruplicate. Lactoferrin has been previously shown to bind AGEs. Li et al. Nature med. 1(10):1057-1061 (1995). Furthermore, lactoferrin was also shown to bind to RAGE Schmidt et al., J. Biol. Chem. 269:9882-9888 (1994), Yan et al., J. Biol. Chem. 269:9889-9897 (1994). However, as is apparent from the Table 1, binding of lactoferrin to RAGE or AGE does not facilitate further binding of AGE-BSA to RAGE. Figure 3 is dose response curve for the formation of RAGE/AGE binding complex in the presence of increasing concentrations of soluble RAGE polypeptide. As shown, increasing concentration of soluble RAGE increased the levels of AGE/RAGE binding until a plateau was reached.

Example 4: Preparation of Antibodies to Human Soluble RAGE

Monoclonal antibodies were generated from mice immunized with the human soluble RAGE extracellular domain expressed in baculovirus as described above.

Hybridoma preparation: Pairs of mice from three strains (Balb/C, Swiss Webster, and RBF/DnJ) were immunized with 100 µg soluble RAGE in complete Hunter's adjuvant, intradermally, on days 0, 7, and 21. Sera were drawn on day 28, and titers tested by EIA of soluble RAGE and FACS analysis of CHO-RAGE transfectants. Two mice were selected as lymphocyte donors, and received 5 µg soluble RAGE IV 72 hours before fusion. Splenocytes from these mice were fused with the mouse myeloma P3X63Ag8.653, and the resultant fusion products were selected with hypoxanthine-aminopterin-thymidine (HAT). Following HAT selection, supernatants from 2100 clones were tested in direct EIA with soluble RAGE. Seventy two strongly positive clones were isolated and then tested for reactivity to CHO RAGE transfectant cells, of which 69 clones were positive. Selected hybridomas were serially subcloned 3X by limiting dilution, and ascites induced in pristane primed IRCF1 mice. The antibodies were purified by affinity chromatography (Protein G) followed by ion exchange chromatography (Q-Sepharose). Purity was tested by reduced SDS-PAGE, and isotype analysis was done by radial immunodiffusion.

The resultant panel was tested for reactivity by EIA, Western blot, and FACS analysis of CHO cells transfected with full length RAGE. The antibodies were also analyzed for epitope variance by competition, and reactivity with RAGE/DCC chimeric protein. Complementary pairs of antibodies were selected,

and antigen capture EIAs specific for human, rat, and mouse RAGE were designed, with sensitivity in the nanogram range.

EIA: Direct antibody capture EIA was done by coating 96-well microtiter plates with 1 μg/well antigen in PBS, and incubating overnight at 4°C. Wells were blocked with PBS/1% BSA or casein, anti-RAGE antibodies added as either neat tissue culture supernatant or 1 μg/well diluted in PBS/1% BSA and incubated for 2 hours at ambient room temperature. After washing 3X with PBS, anti-mouse IgG-alkaline phosphatase was added, and substrate degradation analyzed at 405 nm with a UVMax plate reader.

Antigen capture (sandwich) EIAs were done as above, with the additional step of coating wells with 2 μg anti-RAGE monoclonal antibody diluted in carbonate buffer, pH 9.6, before addition of antigen.

Western Blot: Cell culture supernatants containing human, rat, or mouse RAGE were harvested, and size fractionated on denaturing SDS-PAGE gels. The proteins were transferred to nitrocellulose, incubated with anti-RAGE antibody, and developed using an Amersham detection kit.

FACS analysis: Cells (CHO-RAGE, parental CHO, mouse macrophage, and human smooth muscle aorta) were harvested, and incubated with 5 μg per 10^6 cells anti-RAGE antibody for 45 minutes on ice. After washing with cold PBS, cells were then incubated with anti-mouse IgG-FITC or phycoerythrin for 45 minutes on ice and then washed with PBS. Cells were analyzed for mean channel fluorescence with a Becton-Dickinson FACScan.

Ligand binding analyses and antibody competition studies were done as above, with additional incubations with lactoferrin, and double labelled detection with anti-lactoferrin antibody in parallel with the anti-RAGE antibody.

The direct antibody capture EIA was used as the initial fusion screen to identify positive clones. The assay format was also utilized to determine reactivity with the RAGE chimeric RAGE/DCC protein in which the first immunoglobulin-like domain was replaced with that of DCC. The assay results for the first 20 MAbs are shown in Figure 4. Four MAbs recognized both intact soluble RAGE polypeptide as well as the chimeric protein indicating epitope location outside the first Ig-like domain.

Eleven antibodies were also screened for binding to soluble human RAGE polypeptide fragments as compared to their binding to soluble human RAGE. Figure 4B shows an antigen capture assay utilizing peptide fragment # 2 (WKLNTGRTEAC) (Seq ID NO: 6). The 11 antibodies were also tested for binding to immobilized RAGE in the presence and absence of free soluble RAGE or RAGE polypeptide fragment # 1 (CKGAPKKPPQ) (Seq ID NO: 5), fragment # 2

(WKLNTGRTEAC) (Seq ID NO: 6) and fragment # 8 (GPQDQGTYSC) (Seq ID NO: 7). The results are shown in Figure 4C.

The sandwich EIA using MAbs RBF9D9 (ATCC Accession No. HB-12165) as capture and SW10C1-biotin (ATCC Accession No. HB-12164) as detection is specific for human RAGE, and does not cross react with either rat or mouse RAGE. This assay was used to detect and quantitate the presence of RAGE expressed in baculovirus, as well as CHO cells (Figure 6A). Controls included a concentration range of purified RAGE for generation of a standard curve, as well as RAGE-spiked sera controls; the range of linearity is 20 - 100 ng/well.

A second sandwich EIA, specific for human, rat, and mouse RAGE, uses MAbs RBF9D9 (ATCC Accession No. HB-12165) as capture and SW1E8-biotin (ATCC Accession No. HB-12166) as detection. This assay was used to detect and quantitate recombinant RAGE expressed in insect and mammalian cells (Figure 6B), and sera obtained from lean and obese rats (not shown), including controls as above. Western blot analysis of the antibodies confirms reactivity to RAGE as shown in Figure 5.

Sixty-nine of the 72 anti-RAGE MAbs were positive for binding to cell surface RAGE, as determined by direct immunofluorescent staining of CHO-RAGE transfectant cells. Data from one binding experiment is shown in Figure 7. The other 68 binding MAbs showed similar results. Additional FACS experiments included direct immunofluorescent staining of cells with anti-RAGE MAbs SW10C1 (ATCC Accession No. HB-12164), SW1E8 (ATCC Accession No. HB-12166), and RBF9D9 (ATCC Accession No. HB-12165), all of which strongly recognize cell surface RAGE.

Binding of exogenous lactoferrin, however, was clearly demonstrated on the CHO-RAGE transfectants as shown in Figure 8B. In particular, parental and RAGE transfected CHO cells were separately incubated with 5 μg lactoferrin and stained with both anti-RAGE/phycoerythrin and anti-lactoferrin/FITC. Phycoerythrin fluorescence shown in Figure 8A, indicates the presence of RAGE on the cell surface. FITC fluorescence shown in Figure 8B indicates the binding of lactoferrin to CHO-RAGE transfectants, but not to parental CHO cells.

Epitope analysis: The results of competition experiments, binding to the deletion mutant, and binding to rat and mouse RAGE clearly indicate that the panel of MAbs recognize unique and discrete antigenic determinants. Four MAbs recognize epitopes on the second or third Ig-like domains. One of these four recognizes an epitope that is exposed on the extracellular RAGE, but not cell surface RAGE, perhaps due to proximity to the cell surface. The remaining

69 MAbs recognize epitopes on the first Ig-like domain, and bind to at least four different sites on this domain.

Example 5: Effects of Soluble RAGE on Vascular Permeability

Early changes in vascular permeability are widely recognized as a hallmark of diabetic vascular dysfunction. As a result, the effect of recombinant soluble RAGE was tested in permeability models. See, e.g., Vlassara et al., Laboratory Invest. 70(2):138-151 (1994), Vlassara et al., Proc. Nat'l Acad. Sci. USA 91:11704-11708 (1995).

A. In vitro permeability studies

Bovine aortic endothelial cells (BAEC) were cult-Cured to confluency on nucleopore membranes. Seven days after reaching confluency, cells were incubated for 24 hours with red blood cells (RBCs) isolated from either normal subjects or diabetic patients. Endothelial cells were washed and permeability was measured in a permeability chamber containing minimal essential medium containing 10% fetal calf serum by adding ^{125}I-albumin or ^{3}H-inulin to the upper chamber. The emergence of radioactivity in the lower chamber was then measured over 24 hours at 37°C. Aliquots of medium (5µl) were sampled from the upper and lower chambers every 10 minutes for the first hour and at 1, 2, 4 and 24 hour time points to determine a permeability coefficient.

To determine the effect of recombinant soluble RAGE on in vitro permeability, normal or diabetic RBCs were first incubated with recombinant RAGE or control proteins (VCAM-1)(30µg/ml) before adding to the endothelial cells.

Determination of permeability coefficient (P) was carried out by the following calculation:

$$P=(J)(1/A)(1/(C_t-C_b))$$

where J is the flux of molecules across the filter, A is the surface area of the confluent layer of endothelial cells, C_t is the concentration of tracer in the upper chamber and C_b is the concentration of tracer in the lower chamber. Post-confluent monolayers displaying permeability coefficients greater than 6.5 X 10^{-7}cm/s, for albumin, or greater than 5 X 10^{-6} cm/s for inulin were excluded.

When endothelial cells were exposed to diabetic RBCs, the permeability of the monolayer to both macromolecules and micromolecules increased (Figure 9A and 9B, respectively) compared with endothelial cells exposed

to normal RBCs. This increased permeability was reversed by preincubation of diabetic RBCs with recombinant soluble RAGE, but not with the recombinant soluble control protein (VCAM-1, consisting of the first 3 Ig-like domains of VCAM-1 and having a structure similar to RAGE)(Figure 9A and 9B).

B. TBIR (tissue-blood-isotope ratio) Studies

These studies were performed with normal and diabetic male Wister rats. Diabetes was induced in the rats by intravenous injection of streptozocin (45 mg/Kg) into animals weighing approximately 200 g. Animals were maintained for 9 to 11 weeks post-STZ prior to commencing the TBIR studies. Hyperglycemia (35-40 mmol/liter) was confirmed in diabetic rats.

RBCs were collected from normal or diabetic rats by puncturing the lower abdominal aorta. The RBCs were collected in a solution of dextrose (2.4%), citric acid (2.4%), sodium citrate (0.73%) and 2 parts anticoagulant to 8 parts blood. Blood was centrifuged to remove plasma and buffy coat, and the packed RBCs were washed and infused (4.2×10^9 cells/animal) into normal syngeneic animals (vol. 0.5 ml). After one hour, TBIR was determined by infusion of ^{125}I-albumin followed 30 minutes later by infusion of ^{51}Cr-labelled normal RBCs. Tissue and blood samples were collected 5 minutes later.

To test the effect of recombinant soluble RAGE (and the recombinant control protein), normal or diabetic RBCs were pretreated with the recombinant proteins (60 μg/ml RBCs) prior to infusion. Six animals were used per group.

Pretreatment was carried out as follows: Diabetic rats were infused with either recombinant soluble RAGE or recombinant soluble VCAM-1 (control) (5.15 mg/Kg) 1 hour prior to TBIR measurement. In order to determine the dose of recombinant protein required to achieve a plasma concentration of 30 to 60 μg/ml, pharmacokinetic studies were performed using ^{125}I-labelled recombinant soluble RAGE, the results of which are provided in Figure 10. Radioactivity was measured as the trichloroacetic plasma precipitable fraction. Plasma human recombinant soluble RAGE concentration data were fit to a two compartment open model using nonlinear regression by extended least squares analysis (Siphar, SIMED, Cretail, France). The elimination and distribution half-lives were 26 and 0.13 hours, respectively. For TBIR studies, 10 control diabetic rats were used, 7 diabetic rats were treated with soluble RAGE and 5 rats were treated with the control protein.

TBIR was calculated as a ratio of $[^{125}I]/[^{51}Cr]$ in tissue over the same ratio in blood. One way analysis of variance followed by Dunnet's test was used to

analyze the data for each organ. The results for normal and diabetic rats are given in figures 12A and 12B, respectively.

In normal animals infused with diabetic RBCs, TBIR increased in a number of tissues as compared to infusion of normal RBCs (Figure 11A). Most of the increases in TBIR were prevented by pretreatment of diabetic rats with recombinant soluble RAGE, but not with the control protein, with the exception of kidney tissue, which showed a similar effect with both RAGE and VCAM-1. These results indicate that AGEs on the surface of diabetic RBCs interact with surface RAGE on endothelial cells, triggering activation of the latter cells. This leads to an increased permeability of the endothelial layers. Without being bound to a particular theory, it is believed that pretreatment of diabetic RBCs with soluble RAGE prevents this interaction and, as a result, prevents increases in permeability as demonstrated.

TBIR also increased in various tissues of diabetic rats as compared to those of normal rats. The increase in TBIR observed in some tissues, e.g., kidney, is reversed by acute administration of recombinant soluble RAGE. In other tissues,, e.g., intestine, skin, both recombinant soluble RAGE and control protein had some effects (Figure 11B). TBIR results in diabetic rats were not as clear as those for normal rats. This is believed to be a result of diabetes associated changes in hemodynamic factors that may effect TBIR. Accordingly, additional assays were performed to confirm the efficacy of RAGE in preventing diabetes associated increases in permeability.

C. <u>Albumin Clearance Studies in STZ-induced Diabetic Rats</u>

Young adult Spargue-Dawley rats (male, 6 weeks old, 150 g) were used. Diabetes was again induced by intravenous injection of streptozocin (65 mg/kg, available from Sigma Chemical Co.). Urine output and animal weight were monitored and blood samples collected weekly (tail clip sampling) for determination of serum glucose concentrations (glucose oxidase method). Animals rendered diabetic ([glucose]> 30mM) were used for experimental protocols. Rats were anesthetized with isoflurane (1.5-2%) in the day of the experiment. Cannulae were placed in the jugular veins and left carotid artery. Five percent BSA (6 mg/Kg) was administered to compensate for fluid and protein losses during surgery. The animals were allowed to stabilize for 20-30 minutes prior to treatment.

For animals that received recombinant proteins, the following additional treatments were performed prior to infusion of a tracer: Recombinant

soluble RAGE purified according to the methods described above was administered at infusion rates to achieve plasma concentrations of 60-80 μg/ml for 1.5 hours. Arterial pressure, right atrial pressure, and body temperature were continuously monitored. Western blotting was performed on plasma samples and standards to determine the level of circulating recombinant protein during the studies.

Albumin extravasation (albumin clearance, C_{RSA}) into individual tissues was calculated as the difference between the 35 minute ^{131}I-RSA (rat serum albumin)(injected at t=0) and 5 minute ^{125}I-RSA (injected at t=30 minutes) distribution volumes (See Figure 12). A 30 minute clearance period was chosen in order to minimize loss of extravasated tracer from high permeability tissues (i.e., gut, kidney). Twenty tissue samples were surveyed in the studies: skin (hindlimb and back); skeletal muscle (gastrocnemius, tibialis anterior, abdominal wall); heart (left ventricle); lung (right and lefty lower lobe); lower trachea; aorta, sciatic nerve; retina; kidney; pancreas, jejunum, ileum, colon, testis, cerebrum and visceral fat pad. Initial and final plasma volumes were estimated as the 5 minutes ^{131}I-RSA and ^{125}I-RSA distribution volumes, respectively. Tissue extravascular water contents (EVW) were determiend as (wet weight)-(^{125}I-RSA volume)-(dry weight). Values of C_{RSA} and EVW were normalized to tissue blood-free dry weight. The data were separated into early (2-3 weeks), mid (4-10 weeks), and late (11-20 weeks) phases following streptozocin injection for comparison.

Regression analysis was performed on individual values of C_{RSA} (over 30 minutes) plotted as a function of paired values of EVW. The slope of this relationship approximates the ratio of filtrate to plasma albumin concentration (i.e., the solvent drag coefficient). For hindlimb skin and skeletal muscles, the regression analyses were performed on differences in C_{RSA} and EVW between paired hindlimbs (congested vs. non-congested). The results are shown in Figures 13A (early), 13B (mid) and 13C (late).

Over the 20 week "post-STZ treatment" period, there were increases in albumin clearance in visceral fat (+385%) in the early phase, and in skin (+75%), skeletal muscle (+68%) aorta (80%) and trachea (+84%) in the late phase. No differences in albumin clearance were apparant in intestine, kidney and heart tissue of diabetic rats compared with that of control rats. An intravenous administration of recombinant soluble RAGE (1 mg/Kg body weight) 1 hour prior to the clearance measurement shows some effect on reducing albumin clearance rates in visceral fat in early phase diabetes. Similar results were obtained for tissue extravascular water content per gram of dry weight (Figures 14A-C). Although not shown there were also indications that recombinant soluble RAGE reduced capillary pressure in diabetic rats.

D. Single Microvessel Studies

Normal rats were anesthetized with 1% and the ileal mesentary was exposed through a midline incision. The exposed gut was continuously superfused with mammalian Ringer's solution. Experiments were performed by cannulating a test vessel with control solution (5 mg/ml BSA and washed RBCs from normal rats or from diabetic rats in Ringer's solution) in the absence or presence of recombinant soluble RAGE, and examining the vessel for sticking red blood cells under a microscope. Examples of these vessels are shown in Figures 15A and 15B. Diabetic RBCs adhered to the venular wall when perfused into a normal rat vessel, whereas normal RBCs did not. Subsequent perfusion of diabetic RBCs and recombinant soluble RAGE into the same vessel did not result in further sticking of diabetic RBCs to the vessel wall. Perfusion of diabetic RBCs pretreated with recombinant soluble RAGE into the vessel from the same animal used previously did not result in adherence of RBCs to the vessel wall. Subsequent perfusion of diabetic RBCs (not pretreated with RAGE) did not result in adherence of the RBCs to the vessel wall.

While the foregoing invention has been described in some detail for purposes of clarity and understanding, it will be clear to one skilled in the art from a reading of this disclosure that various changes in form and detail can be made without departing from the true scope of the invention. All publications and patent documents cited in this application are incorporated by reference in their entirety for all purposes to the same extent as if each individual publication or patent document were so individually denoted.

SEQUENCE LISTING

INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1023 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
ATGGCAGCCG GAACAGCAGT TGGAGCCTGG GTGCTGGTCC TCAGTCTGTG GGGGGCAGTA   60
GTAGGTGCTC AAAACATCAC AGCCCGGATT GGCGAGCCAC TGGTGCTGAA GTGTAAGGGG  120
GCCCCCAAGA AACCACCCCA GCGGCTGGAA TGGAAACTGA ACACAGGCCG GACAGAAGCT  180
TGGAAGGTCC TGTCTCCCCA GGGAGGAGGC CCCTGGGACA GTGTGGCTCG TGTCCTTCCC  240
AACGGCTCCC TCTTCCTTCC GGCTGTCGGG ATCCAGGATG AGGGGATTTT CCGGTGCCAG  300
GCAATGAACA GGAATGGAAA GGAGACCAAG TCCAACTACC GAGTCCGTGT CTACCAGATT  360
CCTGGGAAGC CAGAAATTGT AGATTCTGCC TCTGAACTCA CGGCTGGTGT TCCCAATAAG  420
GTGGGGACAT GTGTGTCAGA GGGAAGCTAC CCTGCAGGGA CTCTTAGCTG GCACTTGGAT  480
GGGAAGCCCC TGGTGCCTAA TGAGAAGGGA GTATCTGTGA AGGAACAGAC CAGGAGACAC  540
CCTGAGACAG GGCTCTTCAC ACTGCAGTCG GAGCTAATGG TGACCCCAGC CCGGGGAGGA  600
GATCCCCGTC CCACCTTCTC CTGTAGCTTC AGCCCAGGCC TTCCCCGACA CCGGGCCTTG  660
CGCACAGCCC CCATCCAGCC CCGTGTCTGG GAGCCTGTGC CTCTGGAGGA GGTCCAATTG  720
GTGGTGGAGC CAGAAGGTGG AGCAGTAGCT CCTGGTGGAA CCGTAACCCT GACCTGTGAA  780
GTCCCTGCCC AGCCCTCTCC TCAAATCCAC TGGATGAAGG ATGGTGTGCC CTTGCCCCTT  840
CCCCCCAGCC CTGTGCTGAT CCTCCCTGAG ATAGGGCCTC AGGACCAGGG AACCTACAGC  900
TGTGTGGCCA CCCATTCCAG CCACGGGCCC CAGGAAAGCC GTGCTGTCAG CATCAGCATC  960
ATCGAACCAG GCGAGGAGGG GCCAACTGCA GGCTCTGTGG GAGGATCAGG GCTGGGAACT 1020
```

TGA 1023

INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 340 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Ala Ala Gly Thr Ala Val Gly Ala Trp Val Leu Val Leu Ser Leu
1               5                   10                  15

Trp Gly Ala Val Val Gly Ala Gln Asn Ile Thr Ala Arg Ile Gly Glu
            20                  25                  30

Pro Leu Val Leu Lys Cys Lys Gly Ala Pro Lys Lys Pro Pro Gln Arg
        35                  40                  45

Leu Glu Trp Lys Leu Asn Thr Gly Arg Thr Glu Ala Trp Lys Val Leu
    50                  55                  60

Ser Pro Gln Gly Gly Gly Pro Trp Asp Ser Val Ala Arg Val Leu Pro
65                  70                  75                  80

Asn Gly Ser Leu Phe Leu Pro Ala Val Gly Ile Gln Asp Glu Gly Ile
                85                  90                  95

Phe Arg Cys Gln Ala Met Asn Arg Asn Gly Lys Glu Thr Lys Ser Asn
            100                 105                 110
```

Tyr Arg Val Arg Val Tyr Gln Ile Pro Gly Lys Pro Glu Ile Val Asp
115 120 125

Ser Ala Ser Glu Leu Thr Ala Gly Val Pro Asn Lys Val Gly Thr Cys
130 135 140

Val Ser Glu Gly Ser Tyr Pro Ala Gly Thr Leu Ser Trp His Leu Asp
145 150 155 160

Gly Lys Pro Leu Val Pro Asn Glu Lys Gly Val Ser Val Lys Glu Gln
165 170 175

Thr Arg Arg His Pro Glu Thr Gly Leu Phe Thr Leu Gln Ser Glu Leu
180 185 190

Met Val Thr Pro Ala Arg Gly Gly Asp Pro Arg Pro Thr Phe Ser Cys
195 200 205

Ser Phe Ser Pro Gly Leu Pro Arg His Arg Ala Leu Arg Thr Ala Pro
210 215 220

Ile Gln Pro Arg Val Trp Glu Pro Val Pro Leu Glu Glu Val Gln Leu
225 230 235 240

Val Val Glu Pro Glu Gly Gly Ala Val Ala Pro Gly Gly Thr Val Thr
245 250 255

Leu Thr Cys Glu Val Pro Ala Gln Pro Ser Pro Gln Ile His Trp Met
260 265 270

Lys Asp Gly Val Pro Leu Pro Leu Pro Pro Ser Pro Val Leu Ile Leu
275 280 285

Pro Glu Ile Gly Pro Gln Asp Gln Gly Thr Tyr Ser Cys Val Ala Thr
290 295 300

```
His Ser Ser His Gly Pro Gln Glu Ser Arg Ala Val Ser Ile Ser Ile
305                 310                 315                 320

Ile Glu Pro Gly Glu Glu Gly Pro Thr Ala Gly Ser Val Gly Gly Ser
                325                 330                 335

Gly Leu Gly Thr
            340
```

INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 957 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
GCTCAAAACA TCACAGCCCG GATTGGCGAG CCACTGGTGC TGAAGTGTAA GGGGGCCCCC   60
AAGAAACCAC CCCAGCGGCT GGAATGGAAA CTGAACACAG GCCGGACAGA AGCTTGGAAG  120
GTCCTGTCTC CCCAGGGAGG AGGCCCCTGG GACAGTGTGG CTCGTGTCCT TCCCAACGGC  180
TCCCTCTTCC TTCCCCCTGT CGGGATCCAG GATGAGGGGA TTTTCCGGTG CCAGGCAATG  240
AACAGGAATG GAAAGGAGAC CAAGTCCAAC TACCGAGTCC GTGTCTACCA GATTCCTGGG  300
AAGCCAGAAA TTGTAGATTC TGCCTCTGAA CTCACGGCTG GTGTTCCCAA TAAGGTGGGG  360
ACATGTGTGT CAGAGGGAAG CTACCCTGCA GGGACTCTTA GCTGGCACTT GGATGGGAAG  420
CCCCTGGTGC CTAATGAGAA GGGAGTATCT GTGAAGGAAC AGACCAGGAG ACACCCTGAG  480
ACAGGGCTCT TCACACTGCA GTCGGAGCTA ATGGTGACCC CAGCCCGGGG AGGAGATCCC  540
CGTCCCACCT TCTCCTGTAG CTTCAGCCCA GGCCTTCCCC GACACCGGGC CTTGCGCACA  600
GCCCCCATCC AGCCCCGTGT CTGGGAGCCT GTGCCTCTGG AGGAGGTCCA ATTGGTGGTG  660
GAGCCAGAAG GTGGAGCAGT AGCTCCTGGT GGAACCGTAA CCCTGACCTG TGAAGTCCCT  720
```

GCCCAGCCCT CTCCTCAAAT CCACTGGATG AAGGATGGTG TGCCCTTGCC CCTTCCCCCC 780

AGCCCTGTGC TGATCCTCCC TGAGATAGGG CCTCAGGACC AGGGAACCTA CAGCTGTGTG 840

GCCACCCATT CCAGCCACGG GCCCCAGGAA AGCCGTGCTG TCAGCATCAG CATCATCGAA 900

CCAGGCGAGG AGGGGCCAAC TGCAGGCTCT GTGGGAGGAT CAGGGCTGGG AACTTGA 957

INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 318 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Ala Gln Asn Ile Thr Ala Arg Ile Gly Glu Pro Leu Val Leu Lys Cys
1 5 10 15

Lys Gly Ala Pro Lys Lys Pro Pro Gln Arg Leu Glu Trp Lys Leu Asn
20 25 30

Thr Gly Arg Thr Glu Ala Trp Lys Val Leu Ser Pro Gln Gly Gly Gly
35 40 45

Pro Trp Asp Ser Val Ala Arg Val Leu Pro Asn Gly Ser Leu Phe Leu
50 55 60

Pro Ala Val Gly Ile Gln Asp Glu Gly Ile Phe Arg Cys Gln Ala Met
65 70 75 80

Asn Arg Asn Gly Lys Glu Thr Lys Ser Asn Tyr Arg Val Arg Val Tyr
85 90 95

Gln Ile Pro Gly Lys Pro Glu Ile Val Asp Ser Ala Ser Glu Leu Thr
100 105 110

Ala Gly Val Pro Asn Lys Val Gly Thr Cys Val Ser Glu Gly Ser Tyr
115 120 125

Pro Ala Gly Thr Leu Ser Trp His Leu Asp Gly Lys Pro Leu Val Pro
130 135 140

Asn Glu Lys Gly Val Ser Val Lys Glu Gln Thr Arg Arg His Pro Glu
145 150 155 160

Thr Gly Leu Phe Thr Leu Gln Ser Glu Leu Met Val Thr Pro Ala Arg
165 170 175

Gly Gly Asp Pro Arg Pro Thr Phe Ser Cys Ser Phe Ser Pro Gly Leu
180 185 190

Pro Arg His Arg Ala Leu Arg Thr Ala Pro Ile Gln Pro Arg Val Trp
195 200 205

Glu Pro Val Pro Leu Glu Glu Val Gln Leu Val Val Glu Pro Glu Gly
210 215 220

Gly Ala Val Ala Pro Gly Gly Thr Val Thr Leu Thr Cys Glu Val Pro
225 230 235 240

Ala Gln Pro Ser Pro Gln Ile His Trp Met Lys Asp Gly Val Pro Leu
245 250 255

Pro Leu Pro Pro Ser Pro Val Leu Ile Leu Pro Glu Ile Gly Pro Gln
260 265 270

Asp Gln Gly Thr Tyr Ser Cys Val Ala Thr His Ser Ser His Gly Pro
275 280 285

Gln Glu Ser Arg Ala Val Ser Ile Ser Ile Ile Glu Pro Gly Glu Glu
290 295 300

Gly Pro Thr Ala Gly Ser Val Gly Gly Ser Gly Leu Gly Thr
305 310 315

INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Cys Lys Gly Ala Pro Lys Lys Pro Pro Gln
1 5 10

INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Trp Lys Leu Asn Thr Gly Arg Thr Glu Ala Cys
1 5 10

INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```
Gly Pro Gln Asp Gln Gly Thr Tyr Ser Cys
1               5                   10
```

INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

```
Trp Lys Leu Asn Thr Gly Arg Thr Glu Ala
1               5                   10
```

INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
Cys Glu Val Pro Ala Gln Pro Ser Pro Gln Ile
1               5                   10
```

INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
Cys Arg Ala Met Asn Gln Asn Gly Lys Glu Thr Lys Ser Asn
1               5                   10
```

INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Gly Pro Gln Asp Gln Gly Thr Tyr Ser Cys
1 5 10

INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Ala Gln Asn Ile Thr Ala Arg Ile Gly Glu Pro Leu Val Leu Lys
1 5 10 15

INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Cys Lys Gly Ala Pro Lys Lys Pro Pro Gln
1 5 10

INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```
Glu Gln Thr Arg Arg His Pro Glu Thr
1               5
```

INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

```
Arg Gly Gly Asp Pro Arg Pro Thr Phe Ser Cys
1               5                   10
```

INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

```
Ser Pro Gly Leu Pro Arg His Arg Ala Leu
1               5                   10
```

INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

```
Ser Ser His Gly Pro Gln Glu Ser Arg Ala
1               5                   10
```

INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Ala Gln Asn Ile Thr Ala Arg Ile Gly Glu Pro Leu Val Leu Lys Cys
1 5 10 15

INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Cys Glu Gln Thr Arg Arg His Pro Glu Thr
1 5 10

INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Cys Ser Pro Gly Leu Pro Arg His Arg Ala Leu
1 5 10

INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

```
Ser Ser His Gly Pro Gln Glu Ser Arg Ala Cys
1               5                   10
```

INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

```
GATGGCAGCC GGAACAGCAG TT   22
```

INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

CTCAAGTTCC CAGCCCTGAT CCTC 24

Claims

1. A substantially pure polypeptide, said polypeptide comprising a biologically active, soluble, human RAGE polypeptide.

2. The polypeptide of claim 1, wherein said polypeptide is capable of inhibiting an interaction between an AGE and its receptor.

3. The polypeptide of claim 1, wherein said polypeptide is specifically immunoreactive with an antibody raised against a polypeptide having an amino acid sequence shown in Figure 1A (Seq ID NO: 2) or 1B (Seq ID NOS: 4).

4. The polypeptide of claim 1, wherein said polypeptide comprises the amino acid sequence shown in Figure 1A (Seq ID NO: 2) or 1B (Seq ID NO: 4) or a biologically active fragment thereof.

5. The polypeptide of claim 1, wherein said polypeptide comprises at least ten contiguous amino acids from the amino acid sequence shown in Figure 1A (Seq ID NO: 2) or 1B (Seq ID NO: 4).

6. The soluble human RAGE polyeptide of claim 5, wherein said polypeptide comprises an amino acid sequence selected from the group consisting of: WKLNTGRTEA (Seq ID NO: 8) ; CEVPAQPSPQI (Seq ID NO: 9) ; CRAMNQNGKETKSN (Seq ID NO: 10) ; GPQDQGTYSC (Seq ID NO: 11) ; AQNITARIGEPLVLK (Seq ID NO: 12) ; CKGAPKKPPQ (Seq ID NO: 13) ; EQTRRHPET (Seq ID NO: 14) ; RGGDPRPTFSC (Seq ID NO: 15) ; SPGLPRHRAL (Seq ID NO: 16) ; and SSHGPQESRA (Seq ID NO: 17) .

7. The polypeptide of claim 1, wherein said polypeptide further comprises a labeling group.

8. The polypeptide of claim 7, wherein said labeling group is selected from the group consisting of a radiolabel, a fluorescent group, an enzyme, and a binding group.

9. The polypeptide of claim 1, wherein said polypeptide is coupled to a solid support.

10. A recombinant cell line, said cell line being capable of producing a soluble human RAGE polypeptide or biologically active fragments thereof.

11. The recombinant cell line of claim 10, wherein said cell line is capable of expressing a nucleic acid sequence that is substantially homologous to the nucleic acid sequence shown in Figure 1A (Seq ID NO: 1) or 1B (Seq ID NO: 3).

12. The recombinant cell line of claim 10, wherein said cell line is capable of expressing the nucleic acid sequence shown in Figure 1A (Seq ID NO: 1) or 1B (Seq ID NO: 3).

13. A pharmaceutical composition, comprising the soluble human RAGE polypeptide in combination with a pharmaceutically acceptable carrier.

14. The pharmaceutical composition of claim 13, wherein said soluble human RAGE polypeptide is capable of inhibiting an interaction between an AGE and its receptor.

15. The pharmaceutical composition of claim 13, wherein said soluble human RAGE polypeptide comprises the amino acid sequence shown in Figure 1A (Seq ID NO: 2) or 1B (Seq ID NO: 4) or a biologically active fragment thereof.

16. The pharmaceutical composition of claim 13, wherein said soluble human RAGE polypeptide comprises an amino acid sequence selected from the group consisting of: WKLNTGRTEA (Seq ID NO: 8) ; CEVPAQPSPQI (Seq ID NO: 9) ; CRAMNQNGKETKSN (Seq ID NO: 10) ; GPQDQGTYSC (Seq ID NO: 11) ; AQNITARIGEPLVLK (Seq ID NO: 12) ; CKGAPKKPPQ (Seq ID NO: 13) ; EQTRRHPET (Seq ID NO: 14) ; RGGDPRPTFSC (Seq ID NO: 15) ; SPGLPRHRAL (Seq ID NO: 16) ; and SSHGPQESRA (Seq ID NO: 17) .

17. A method of purifying an AGE from a mixture of proteins containing an AGE, said method comprising the steps of:
immobilizing a soluble human RAGE polypeptide on a solid support;

contacting said immobilized RAGE polypeptide with the mixture of proteins containing an AGE whereby said RAGE polypeptide selectively binds said AGE in said mixture of proteins;
removing unbound proteins from said immobilized RAGE polypeptide;
and isolating purified AGE which was selectively bound to said immobilized RAGE.

18. A method of screening a test compound to determine if said test compound is an agonist or antagonist of a AGE/RAGE interaction, the method comprising the steps of:
separately incubating a soluble human RAGE polypeptide with an AGE in the presence and absence of said test compound;
detecting a level of interaction between said soluble human RAGE polypeptide and said AGE in the presence and absence of said test compound;
and comparing said level of interaction in the presence and absence of said test compound, an increase or decrease in said level of interaction in the presence of said test compound being indicative that said test compound is an agonist or antagonist of said interaction, respectively.

19. The method of claim 18, wherein one of said RAGE polypeptide or AGE is bound to a solid support and the other is labeled with a labeling group.

20. The method of claim 19, wherein said labeling group is selected from the group consisting of a radiolabel, a fluoresent label, an enzymatic label and an antibody epitope.

21. A method of inhibiting an interaction between an AGE and a receptor which specifically binds said AGE, said method comprising contacting said AGE with an effective amount of a soluble human RAGE polypeptide.

22. The method of claim 21, wherein said receptor is RAGE.

23. A method of treating a patient for symptoms of a disorder, wherein said symptoms are caused by an interaction between an AGE and its receptor, the method comprising administering to said patient an effective amount of a soluble human RAGE polypeptide.

24. The method of claim 23, wherein said patient is a diabetic patient, and said symptom is increased vascular permeability.

25. The method of claim 23, wherein said disorder is Diabetes Mellitus and said symptom is selected from the group consisting of diabetic microvasculopathy, diabetic macrovasculopathy and occlusive vascular disorder.

26. The method of claim 25, wherein said symptom is selected from neuropathy, nephropathy, retinopathy and atherosclerosis.

27. The method of claim 23, wherein said symptom is hemodialysis-associated amyloidosis.

28. An isolated nucleic acid sequence, said nucleic acid sequence encoding a soluble human RAGE polypeptide.

29. The isolated nucleic acid sequence of claim 28, wherein said nucleic acid sequence encodes an amino acid sequence shown in Figure 1A (Seq ID NO: 2) or 1B (Seq ID NO: 4), or a biologically active fragment thereof.

30. The isolated nucleic acid sequence of claim 28, which is substantially homologous to a nucleic acid sequence shown in Figure 1A (Seq ID NO: 1) or 1B (Seq ID NO: 3).

31. The isolated nucleic acid sequence of claim 28, which comprises the nucleic acid sequence shown in Figure 1A (Seq ID NO: 1) or 1B (Seq ID NO: 3).

32. A screening kit, comprising a soluble human RAGE polypeptide and an AGE one of said RAGE polypeptide or said AGE being immobilized on a solid support and the other of said RAGE polypeptide or said AGE comprising a chemical label.

33. The screening kit of claim 32, wherein said soluble human RAGE polypeptide comprises an amino acid sequence that is substantially homologous to the amino acid sequence shown in Figure 1A (Seq ID NO: 2) or 1B (Seq ID NO: 4), or a biologically active fragment thereof.

34. The screening kit of claim 32, wherein said soluble human RAGE polypeptide comprises an amino acid sequence shown in Figure 1A (Seq ID NO: 2) or 1B (Seq ID NO: 4).

35. The screening kit of claim 32, wherein said AGE is AGE-BSA.

36. Use of an effective amount of a soluble human RAGE polypeptide for the production of a pharmaceutical compound or composition for the treatment of a patient for symtoms of a disorder, wherein said symptoms are caused by an interaction between an AGE and its receptor.

37. Use according to claim 36, wherein said patient is a diabetic patient, and said symptom is increased vascular permeability.

38. Use according to claim 36, wherein said disorder is Diabetes Mellitus and said symptom is selected from the group consisting of diabetic microvasculopathy, diabetic macrovasculopathy and occlusive vascular disorder.

39. Use according to claim 38, wherein said symptom is selected from neuropathy, nephropathy, retinopathy and atherosclerosis.

40. Use according to claim 36, wherein said symptom is hemodialysis-associated amyloidosis.

```
     MetAlaAlaGlyThrAlaValGlyAlaTrpValLeuValLeuSerLeuTrpGlyAlaVal
   1 ATGGCAGCCGGAACAGCAGTTGGAGCCTGGGTGCTGGTCCTCAGTCTGTGGGGGGCAGTA

     ValGlyAlaGlnAsnIleThrAlaArgIleGlyGluProLeuValLeuLysCysLysGly
  61 GTAGGTGCTCAAAACATCACAGCCCGGATTGGCGAGCCACTGGTGCTGAAGTGTAAGGGG

     AlaProLysLysProProGlnArgLeuGluTrpLysLeuAsnThrGlyArgThrGluAla
 121 GCCCCCAAGAAACCACCCCAGCGGCTGGAATGGAAACTGAACACAGGCCGGACAGAAGCT

     TrpLysValLeuSerProGlnGlyGlyGlyProTrpAspSerValAlaArgValLeuPro
 181 TGGAAGGTCCTGTCTCCCCAGGGAGGAGGCCCCTGGGACAGTGTGGCTCGTGTCCTTCCC

     AsnGlySerLeuPheLeuProAlaValGlyIleGlnAspGluGlyIlePheArgCysGln
 241 AACGGCTCCCTCTTCCTTCCGGCTGTCGGGATCCAGGATGAGGGGATTTTCCGGTGCCAG

     AlaMetAsnArgAsnGlyLysGluThrLysSerAsnTyrArgValArgValTyrGlnIle
 301 GCAATGAACAGGAATGGAAAGGAGACCAAGTCCAACTACCGAGTCCGTGTCTACCAGATT

     ProGlyLysProGluIleValAspSerAlaSerGluLeuThrAlaGlyValProAsnLys
 361 CCTGGGAAGCCAGAAATTGTAGATTCTGCCTCTGAACTCACGGCTGGTGTTCCCAATAAG

     ValGlyThrCysValSerGluGlySerTyrProAlaGlyThrLeuSerTrpHisLeuAsp
 421 GTGGGGACATGTGTGTCAGAGGGAAGCTACCCTGCAGGGACTCTTAGCTGGCACTTGGAT

     GlyLysProLeuValProAsnGluLysGlyValSerValLysGluGlnThrArgArgHis
 481 GGGAAGCCCCTGGTGCCTAATGAGAAGGGAGTATCTGTGAAGGAACAGACCAGGAGACAC

     ProGluThrGlyLeuPheThrLeuGlnSerGluLeuMetValThrProAlaArgGlyGly
 541 CCTGAGACAGGGCTCTTCACACTGCAGTCGGAGCTAATGGTGACCCCAGCCCGGGGAGGA

     AspProArgProThrPheSerCysSerPheSerProGlyLeuProArgHisArgAlaLeu
 601 GATCCCCGTCCCACCTTCTCCTGTAGCTTCAGCCCAGGCCTTCCCCGACACCGGGCCTTG

     ArgThrAlaProIleGlnProArgValTrpGluProValProLeuGluGluValGlnLeu
 661 CGCACAGCCCCCATCCAGCCCCGTGTCTGGGAGCCTGTGCCTCTGGAGGAGGTCCAATTG

     ValValGluProGluGlyGlyAlaValAlaProGlyGlyThrValThrLeuThrCysGlu
 721 GTGGTGGAGCCAGAAGGTGGAGCAGTAGCTCCTGGTGGAACCGTAACCCTGACCTGTGAA

     ValProAlaGlnProSerProGlnIleHisTrpMetLysAspGlyValProLeuProLeu
 781 GTCCCTGCCCAGCCCTCTCCTCAAATCCACTGGATGAAGGATGGTGTGCCCTTGCCCCTT

     ProProSerProValLeuIleLeuProGluIleGlyProGlnAspGlnGlyThrTyrSer
 841 CCCCCCAGCCCTGTGCTGATCCTCCCTGAGATAGGGCCTCAGGACCAGGGAACCTACAGC

     CysValAlaThrHisSerSerHisGlyProGlnGluSerArgAlaValSerIleSerIle
 901 TGTGTGGCCACCCATTCCAGCCACGGGCCCCAGGAAAGCCGTGCTGTCAGCATCAGCATC

     IleGluProGlyGluGluGlyProThrAlaGlySerValGlyGlySerGlyLeuGlyThr
 961 ATCGAACCAGGCGAGGAGGGGCCAACTGCAGGCTCTGTGGGAGGATCAGGGCTGGGAACT

     OP
1021 TGA
```

Figure 1A

```
    AlaGlnAsnIleThrAlaArgIleGlyGluProLeuValLeuLysCysLysGlyAlaPro
  1 GCTCAAAACATCACAGCCCGGATTGGCGAGCCACTGGTGCTGAAGTGTAAGGGGGCCCCC

    LysLysProProGlnArgLeuGluTrpLysLeuAsnThrGlyArgThrGluAlaTrpLys
 61 AAGAAACCACCCCAGCGGCTGGAATGGAAACTGAACACAGGCCGGACAGAAGCTTGGAAG

    ValLeuSerProGlnGlyGlyGlyProTrpAspSerValAlaArgValLeuProAsnGly
121 GTCCTGTCTCCCCAGGGAGGAGGCCCCTGGGACAGTGTGGCTCGTGTCCTTCCCAACGGC

    SerLeuPheLeuProAlaValGlyIleGlnAspGluGlyIlePheArgCysGlnAlaMet
181 TCCCTCTTCCTTCCGGCTGTCGGGATCCAGGATGAGGGGATTTTCCGGTGCCAGGCAATG

    AsnArgAsnGlyLysGluThrLysSerAsnTyrArgValArgValTyrGlnIleProGly
241 AACAGGAATGGAAAGGAGACCAAGTCCAACTACCGAGTCCGTGTCTACCAGATTCCTGGG

    LysProGluIleValAspSerAlaSerGluLeuThrAlaGlyValProAsnLysValGly
301 AAGCCAGAAATTGTAGATTCTGCCTCTGAACTCACGGCTGGTGTTCCCAATAAGGTGGGG

    ThrCysValSerGluGlySerTyrProAlaGlyThrLeuSerTrpHisLeuAspGlyLys
361 ACATGTGTGTCAGAGGGAAGCTACCCTGCAGGGACTCTTAGCTGGCACTTGGATGGGAAG

    ProLeuValProAsnGluLysGlyValSerValLysGluGlnThrArgArgHisProGlu
421 CCCCTGGTGCCTAATGAGAAGGGAGTATCTGTGAAGGAACAGACCAGGAGACACCCTGAG

    ThrGlyLeuPheThrLeuGlnSerGluLeuMetValThrProAlaArgGlyGlyAspPro
481 ACAGGGCTCTTCACACTGCAGTCGGAGCTAATGGTGACCCCAGCCCGGGGAGGAGATCCC

    ArgProThrPheSerCysSerPheSerProGlyLeuProArgHisArgAlaLeuArgThr
541 CGTCCCACCTTCTCCTGTAGCTTCAGCCCAGGCCTTCCCCGACACCGGGCCTTGCGCACA

    AlaProIleGlnProArgValTrpGluProValProLeuGluGluValGlnLeuValVal
601 GCCCCCATCCAGCCCCGTGTCTGGGAGCCTGTGCCTCTGGAGGAGGTCCAATTGGTGGTG

    GluProGluGlyGlyAlaValAlaProGlyGlyThrValThrLeuThrCysGluValPro
661 GAGCCAGAAGGTGGAGCAGTAGCTCCTGGTGGAACCGTAACCCTGACCTGTGAAGTCCCT

    AlaGlnProSerProGlnIleHisTrpMetLysAspGlyValProLeuProLeuProPro
721 GCCCAGCCCTCTCCTCAAATCCACTGGATGAAGGATGGTGTGCCCTTGCCCCTTCCCCCC

    SerProValLeuIleLeuProGluIleGlyProGlnAspGlnGlyThrTyrSerCysVal
781 AGCCCTGTGCTGATCCTCCCTGAGATAGGGCCTCAGGACCAGGGAACCTACAGCTGTGTG

    AlaThrHisSerSerHisGlyProGlnGluSerArgAlaValSerIleSerIleIleGlu
841 GCCACCCATTCCAGCCACGGGCCCCAGGAAAGCCGTGCTGTCAGCATCAGCATCATCGAA

    ProGlyGluGluGlyProThrAlaGlySerValGlyGlySerGlyLeuGlyThrOP
901 CCAGGCGAGGAGGGGCCAACTGCAGGCTCTGTGGGAGGATCAGGGCTGGGAACTTGA
```

Figure 1B

Indirect Solid-phase RAGE/AGE binding Assay

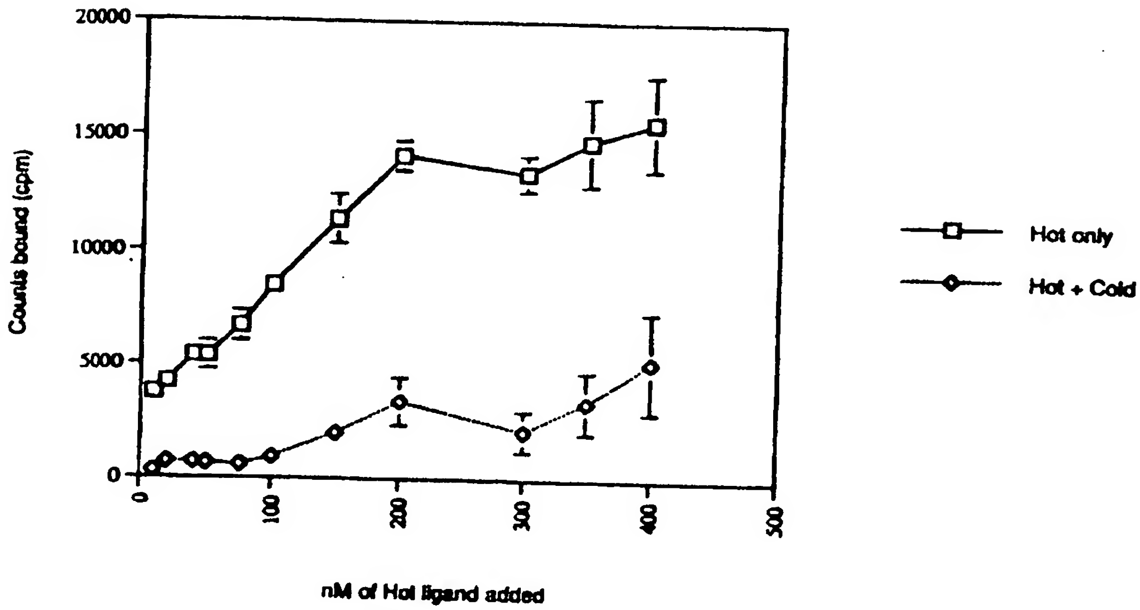


Figure 2A

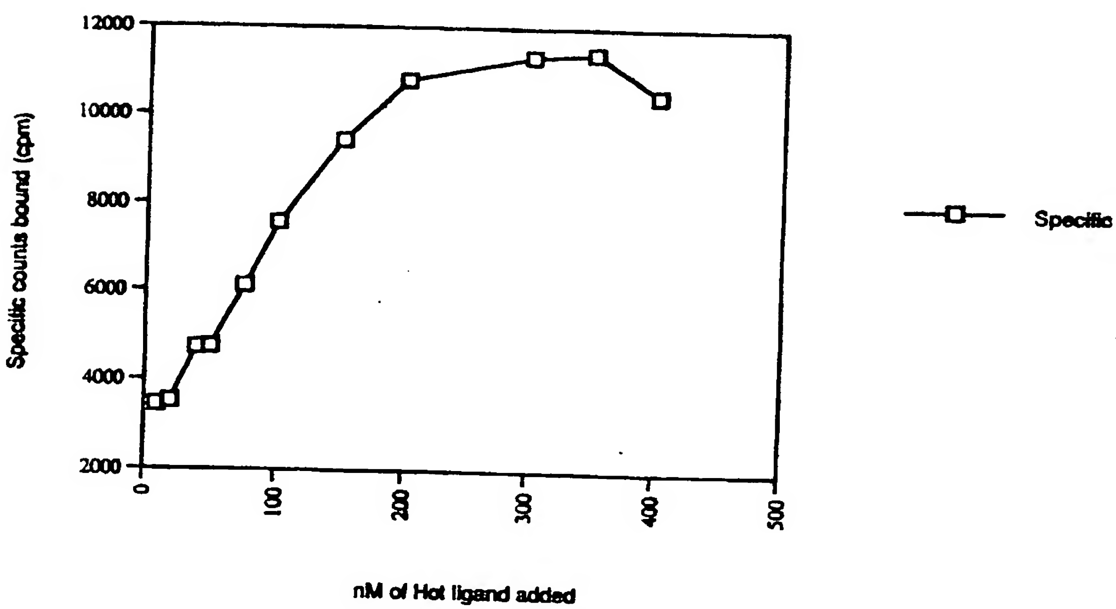


Figure 2B

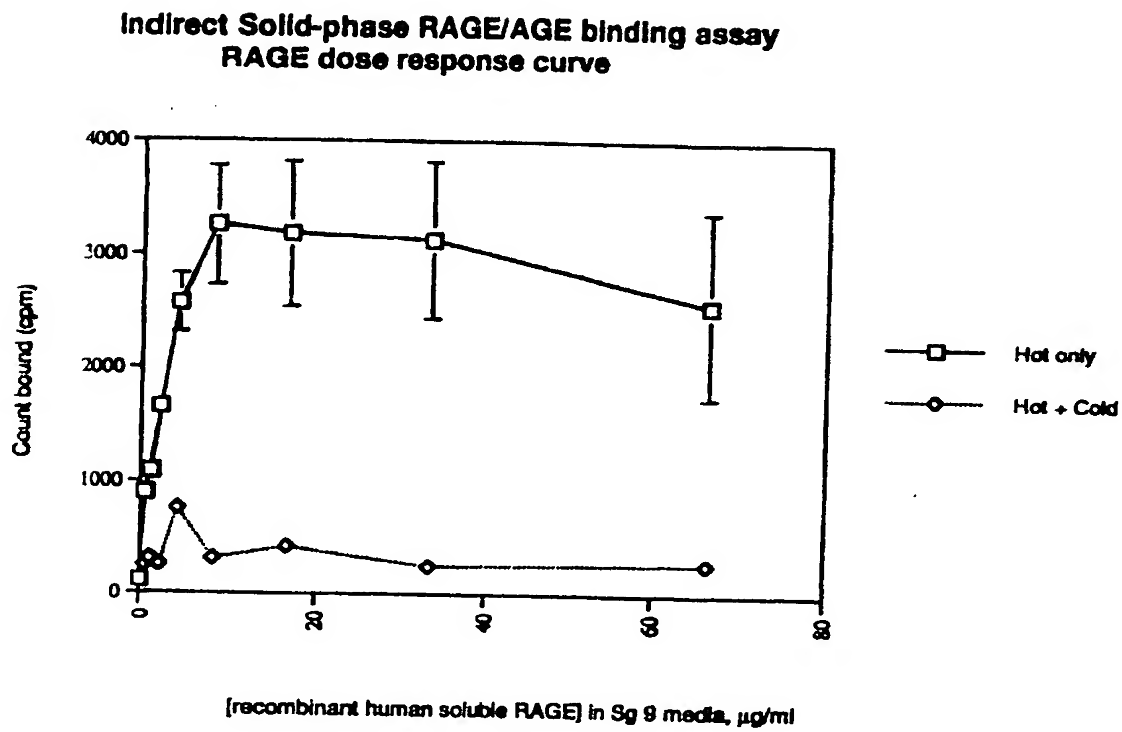

Indirect Solid-phase RAGE/AGE binding assay
RAGE dose response curve
Count bound (cpm)
4000
3000
2000
1000
0
0
20
40
60
80
[recombinant human soluble RAGE] in Sg 9 media, µg/ml
Hot only
Hot + Cold

Figure 3

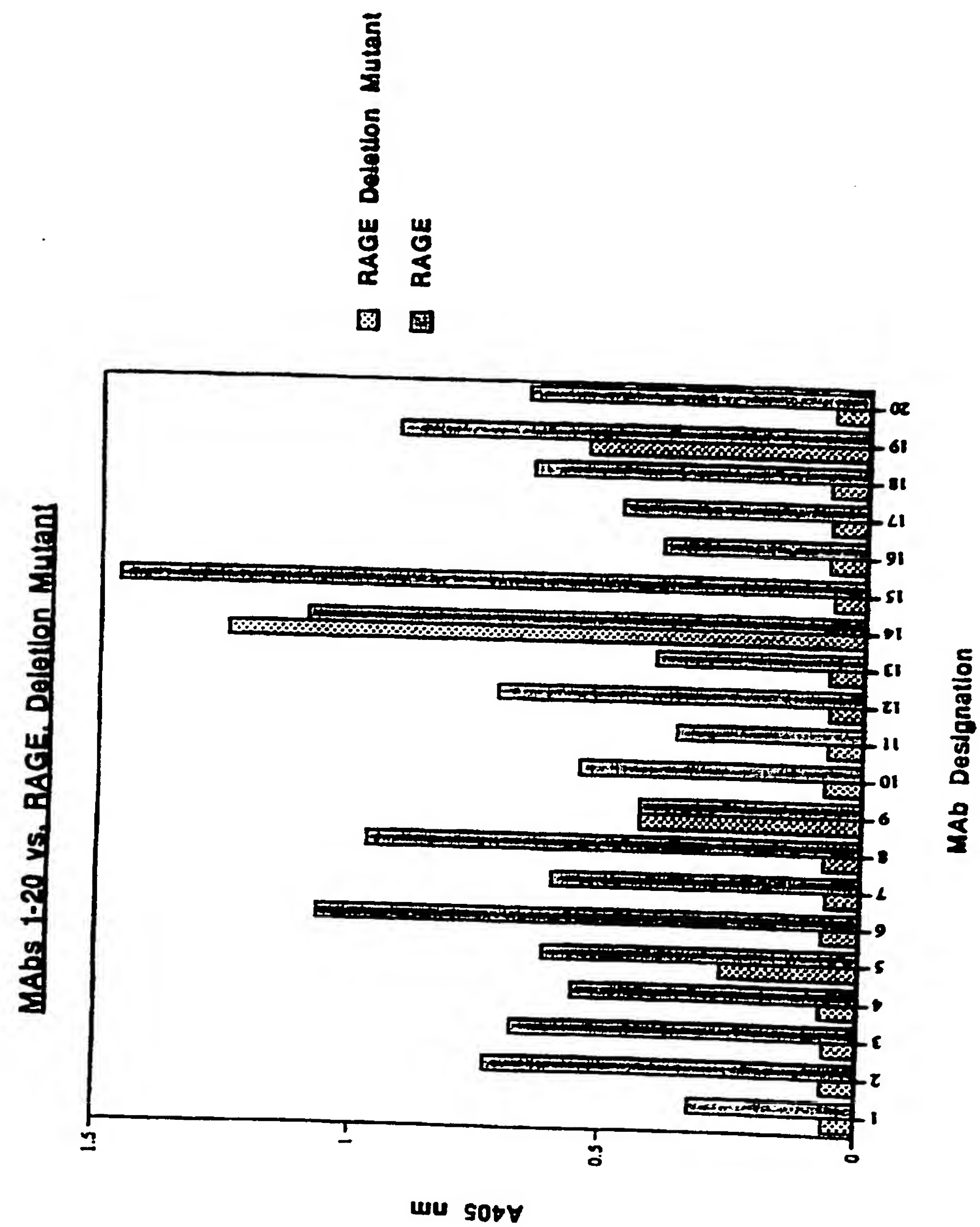

MAbs 1-20 vs. RAGE, Deletion Mutant
RAGE Deletion Mutant
RAGE
A405 nm
0
0.5
1
1.5
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
MAb Designation

Figure 4A

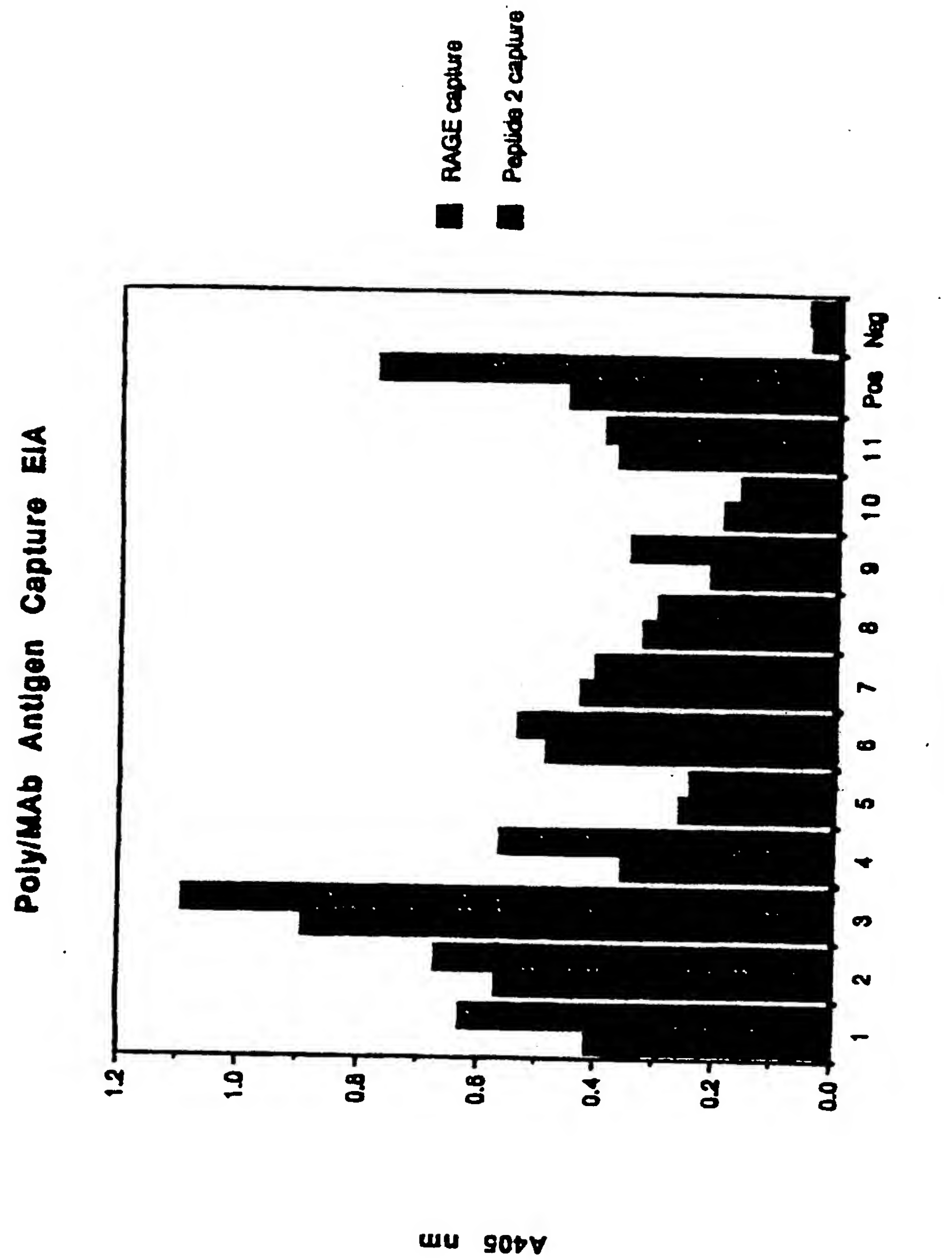

Poly/MAb Antigen Capture EIA
RAGE capture
Peptide 2 capture
1.2
1.0
0.8
0.6
0.4
0.2
0.0
A405 nm
1
2
3
4
5
6
7
8
9
10
11
Pos
Neg
MAb Designation

Figure 4B

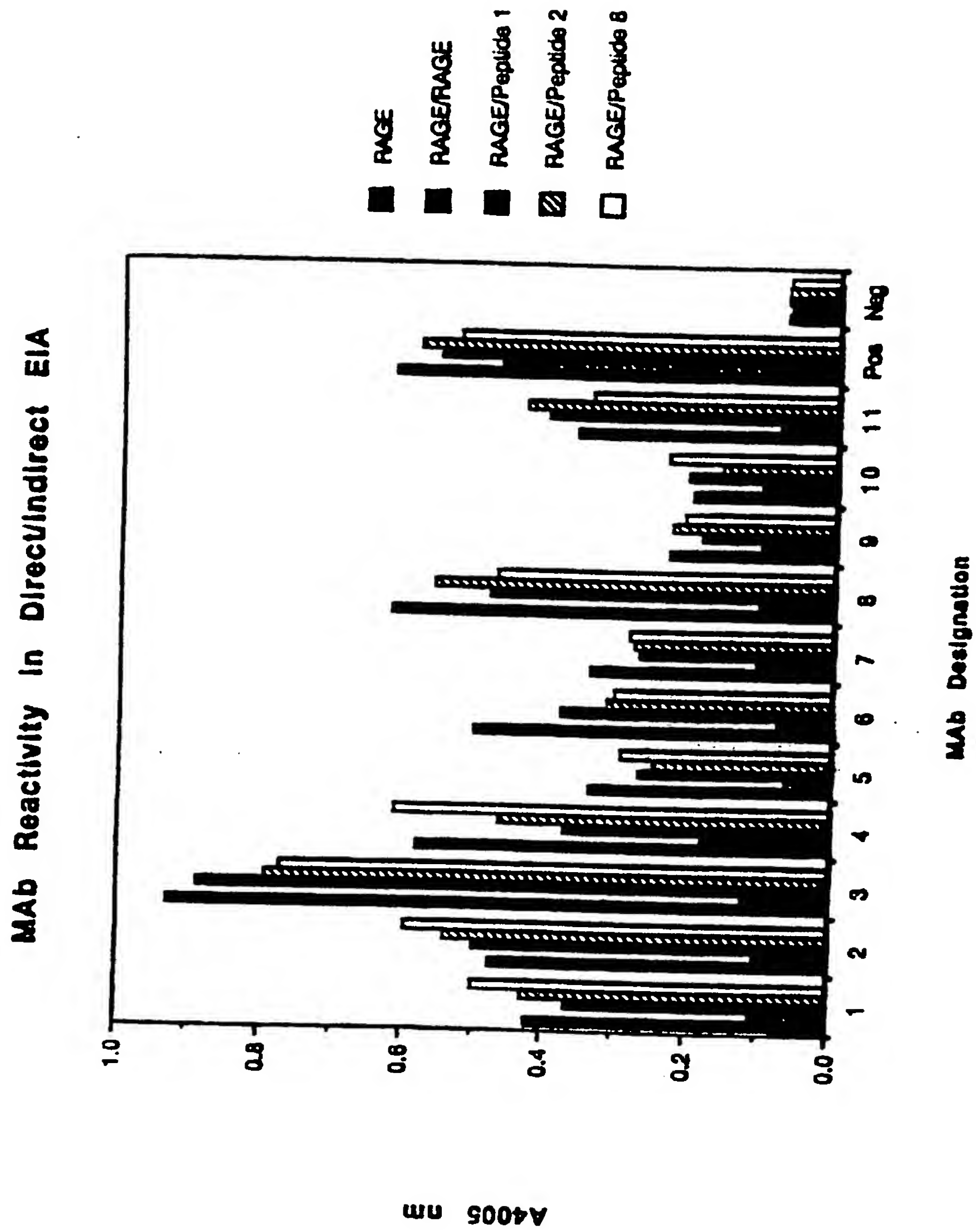

MAb Reactivity In Direct/Indirect EIA
RAGE
RAGE/RAGE
RAGE/Peptide 1
RAGE/Peptide 2
RAGE/Peptide 8
A4005 nm
1.0
0.8
0.6
0.4
0.2
0.0
1
2
3
4
5
6
7
8
9
10
11
Pos
Neg
MAb Designation

Figure 4C

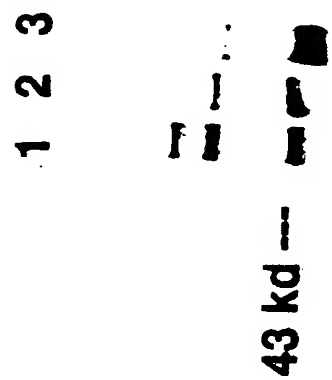


Figure 5

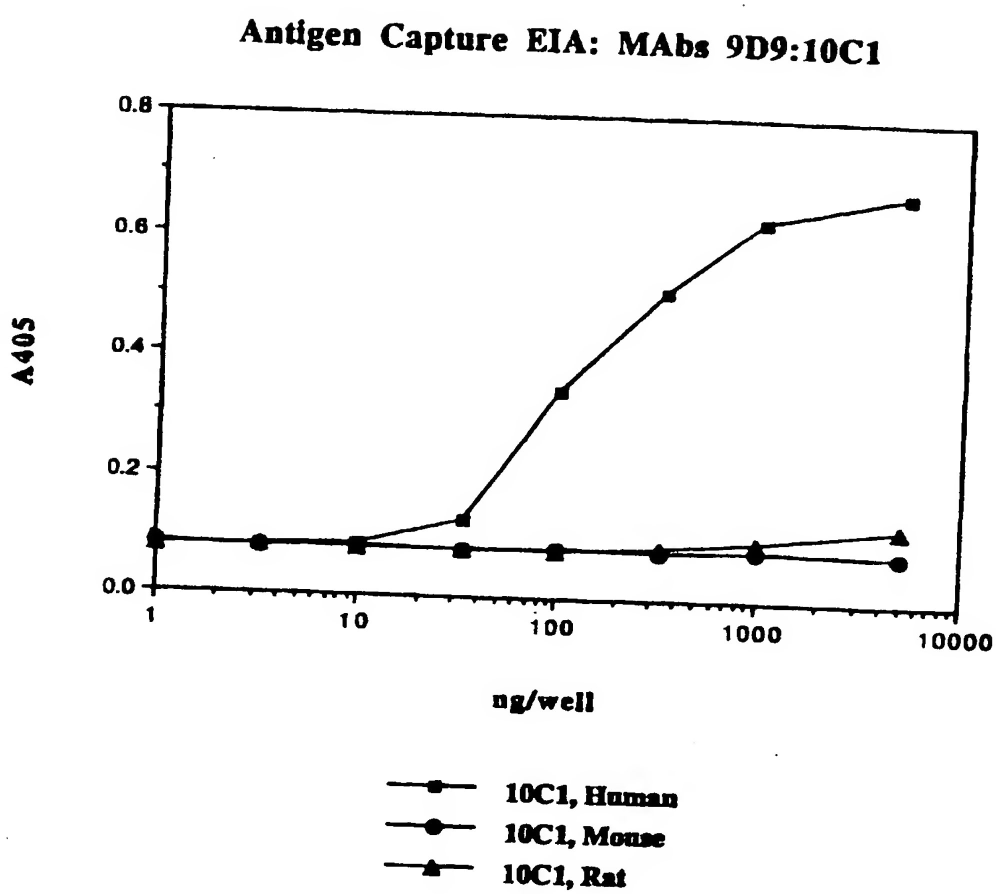

Antigen Capture EIA: MAbs 9D9:10C1
0.8
0.6
0.4
0.2
0.0
A405
1
10
100
1000
10000
ng/well
10C1, Human
10C1, Mouse
10C1, Rat

Figure 6A

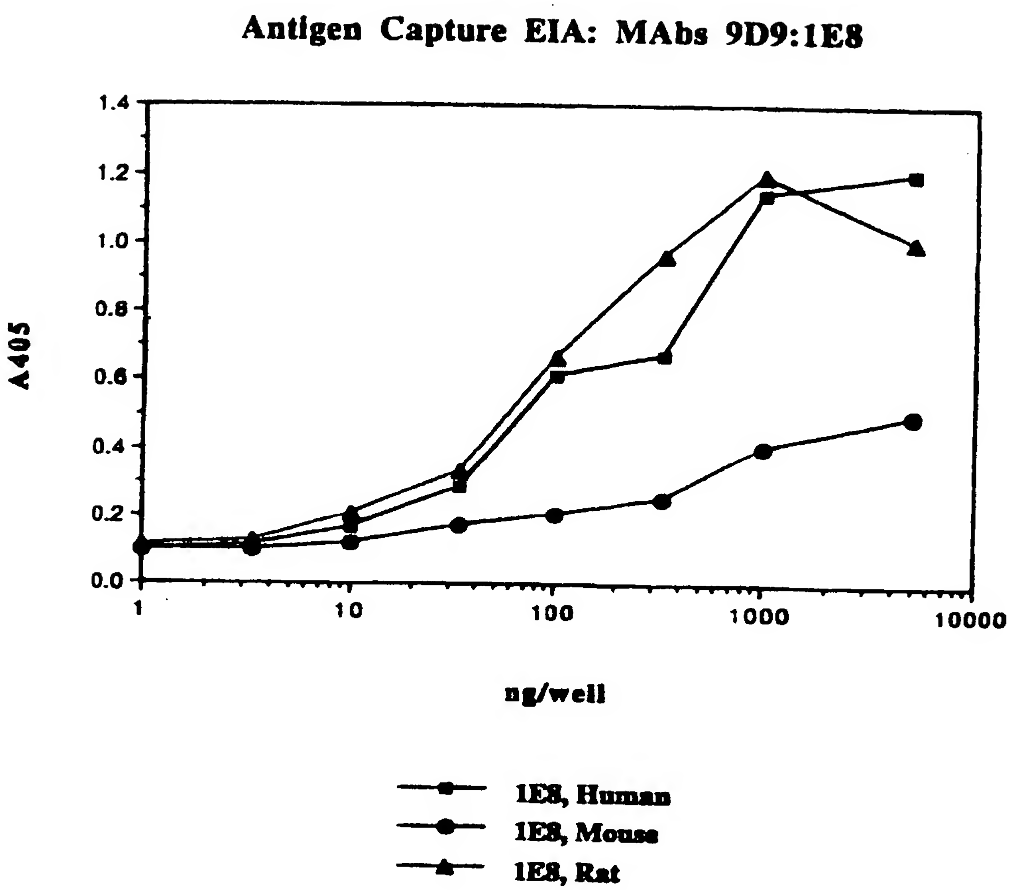

Antigen Capture EIA: MAbs 9D9:1E8
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
A405
1
10
100
1000
10000
ng/well
1E8, Human
1E8, Mouse
1E8, Rat

Figure 6B

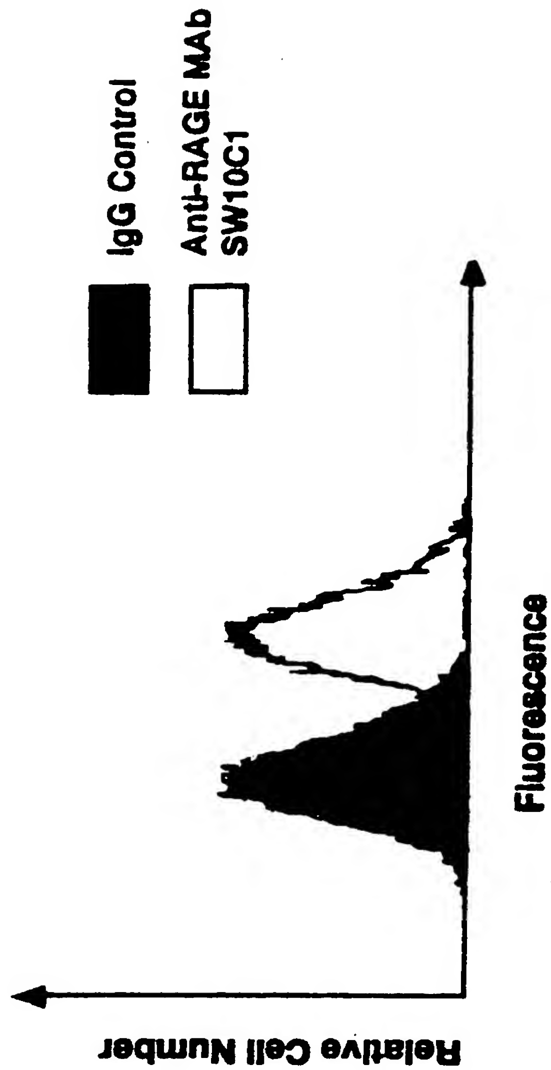

IgG Control
Anti-RAGE MAb
SW10C1
Fluorescence
Relative Cell Number

Figure 7

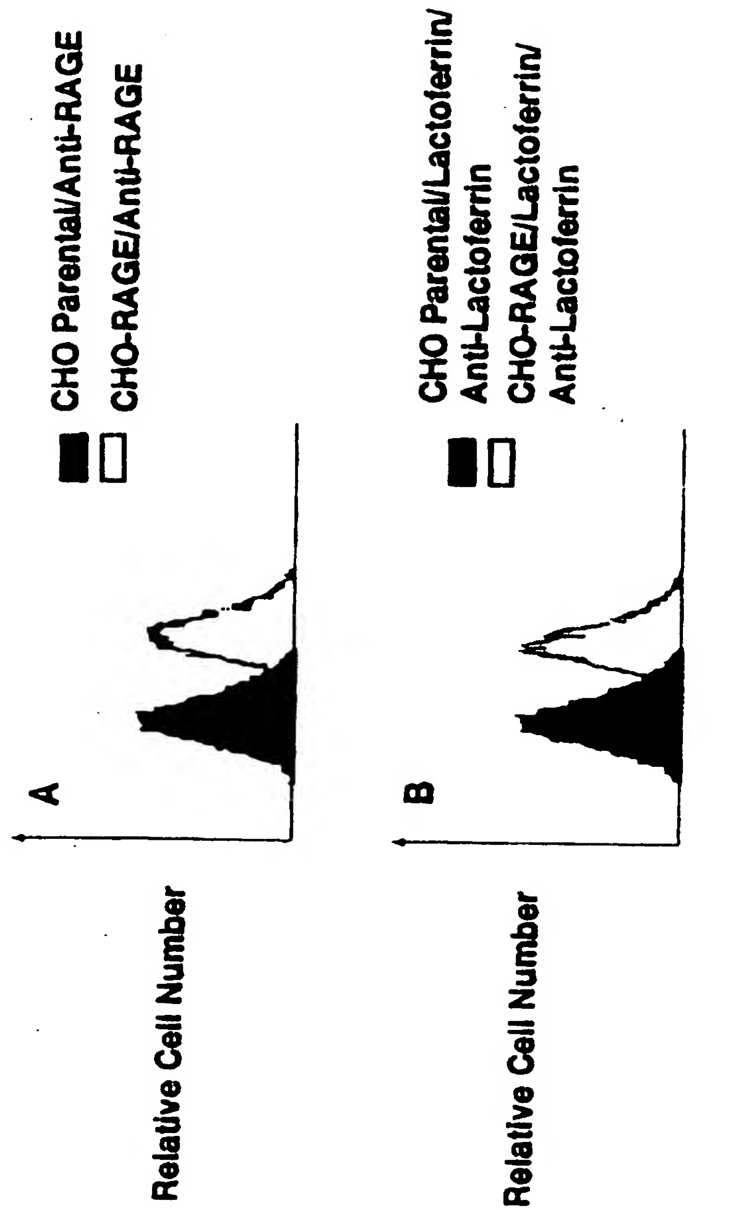

A
CHO Parental/Anti-RAGE
CHO-RAGE/Anti-RAGE
Relative Cell Number
B
CHO Parental/Lactoferrin/
Anti-Lactoferrin
CHO-RAGE/Lactoferrin/
Anti-Lactoferrin
Relative Cell Number

Figure 8

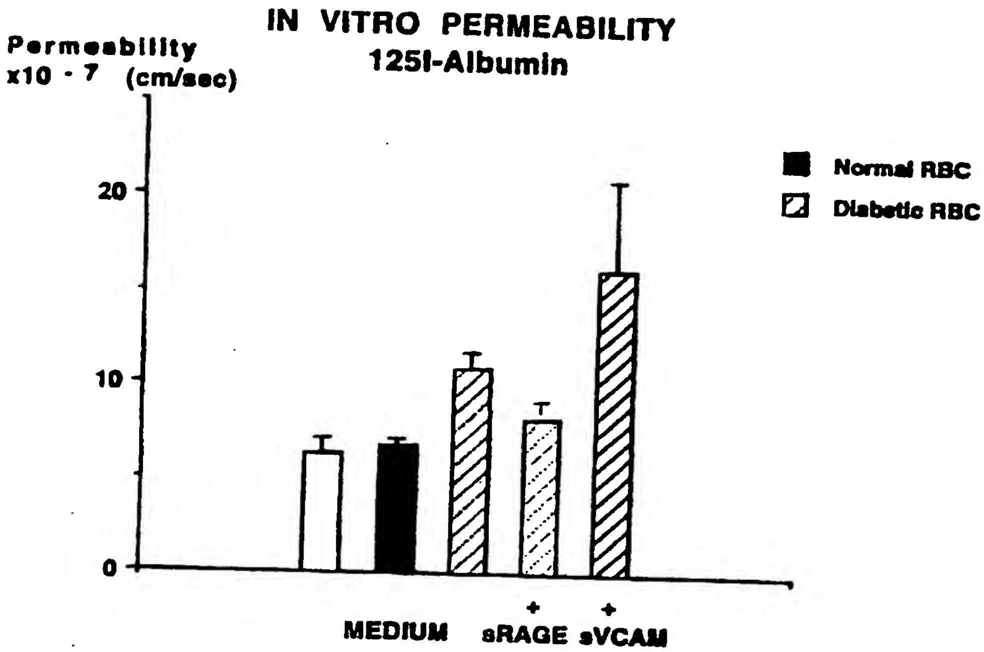

IN VITRO PERMEABILITY
125I-Albumin
Permeability
x10 - 7 (cm/sec)
20
10
0
Normal RBC
Diabetic RBC
MEDIUM
+
sRAGE
+
sVCAM

Figure 9A

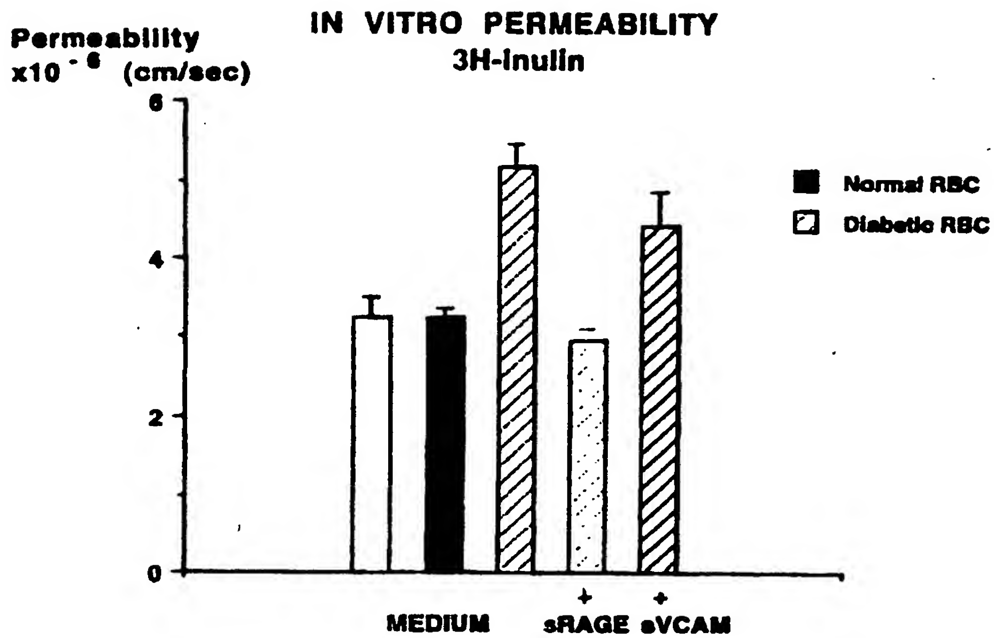

IN VITRO PERMEABILITY
3H-Inulin
Permeability
x10⁻⁶ (cm/sec)
6
4
2
0
Normal RBC
Diabetic RBC
MEDIUM
+
sRAGE
+
sVCAM

Figure 9B

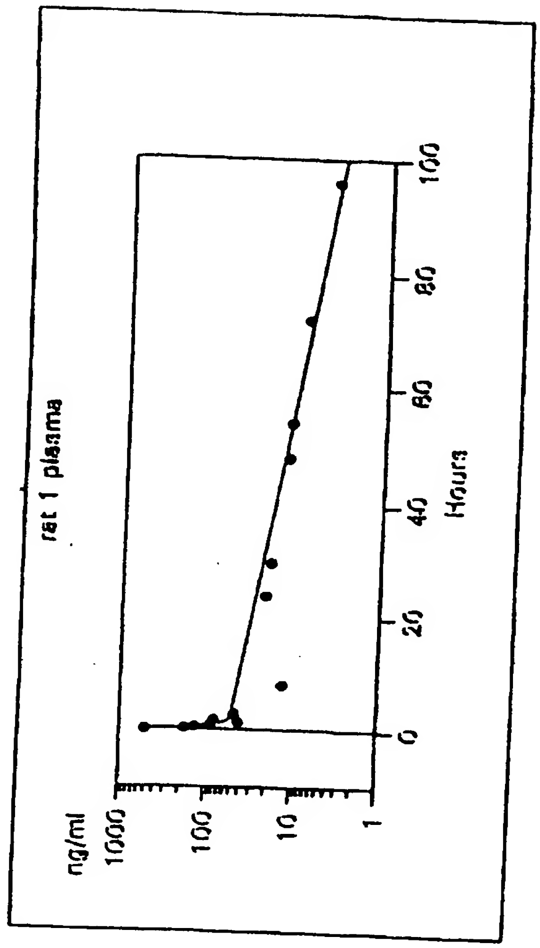


Figure 10

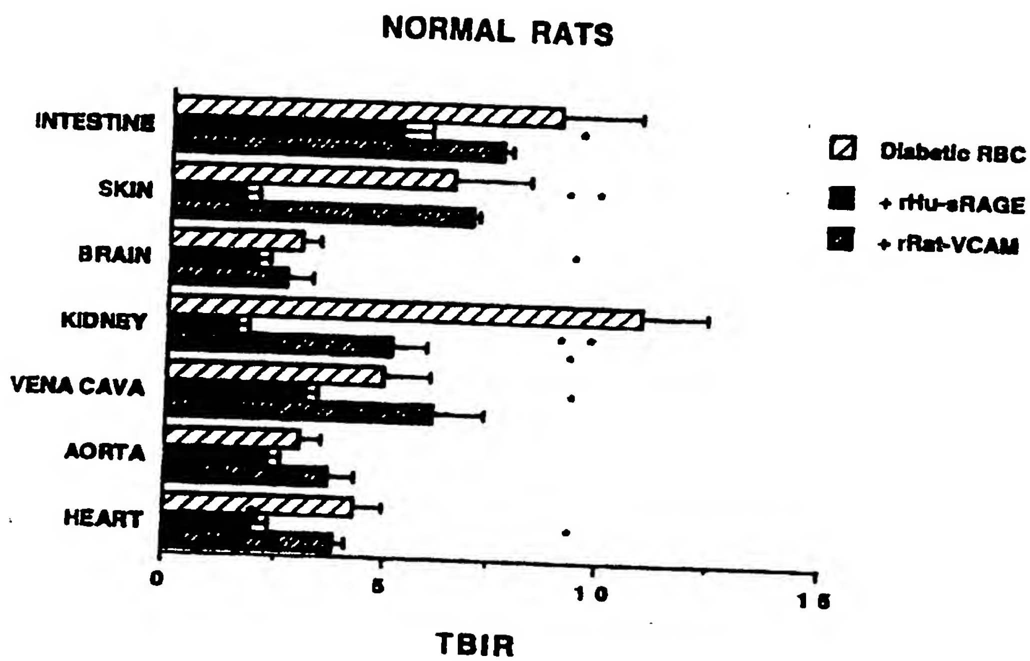

NORMAL RATS
INTESTINE
SKIN
BRAIN
KIDNEY
VENA CAVA
AORTA
HEART
0
5
10
15
TBIR
Diabetic RBC
+ rHu-sRAGE
+ rRat-VCAM

Figure 11A

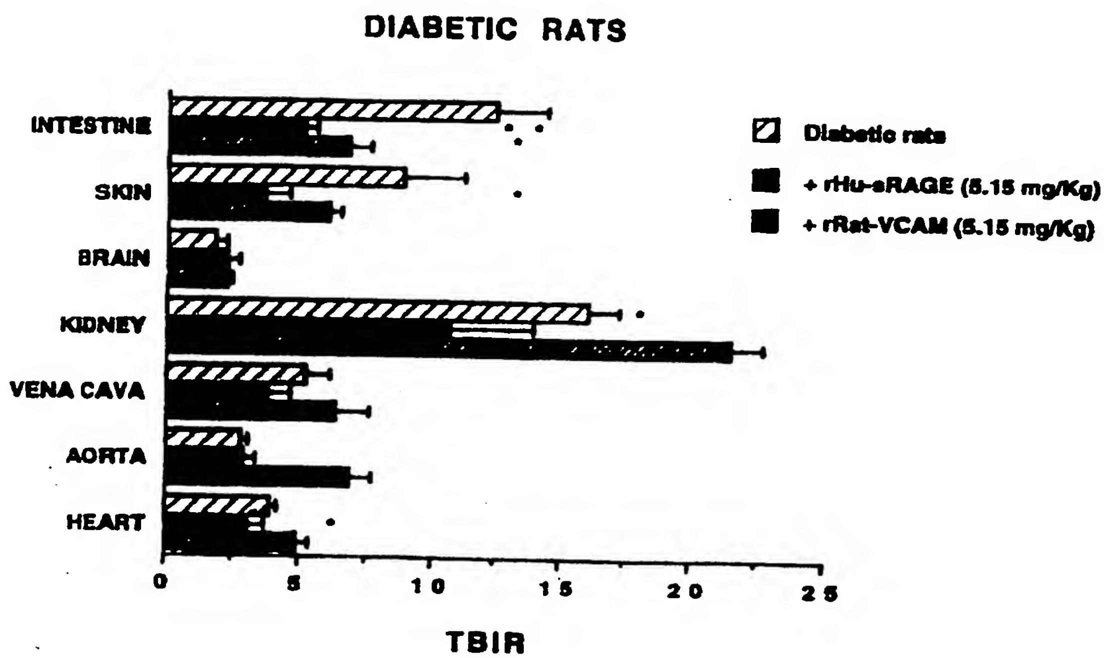

DIABETIC RATS
INTESTINE
SKIN
BRAIN
KIDNEY
VENA CAVA
AORTA
HEART
Diabetic rats
+ rHu-sRAGE (5.15 mg/Kg)
+ rRat-VCAM (5.15 mg/Kg)
0
5
10
15
20
25
TBIR

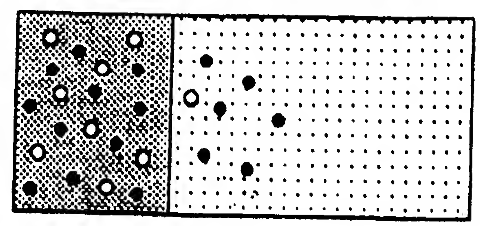
Figure 11B

BLOOD - TISSUE ALBUMIN TRANSPORT

TRACER UPTAKE METHOD

- ● test tracer (^{131}I-RSA) *circulation time = 35 min*
- ○ reference (^{125}I-RSA) *circulation time = 5 min*

TISSUE SAMPLE

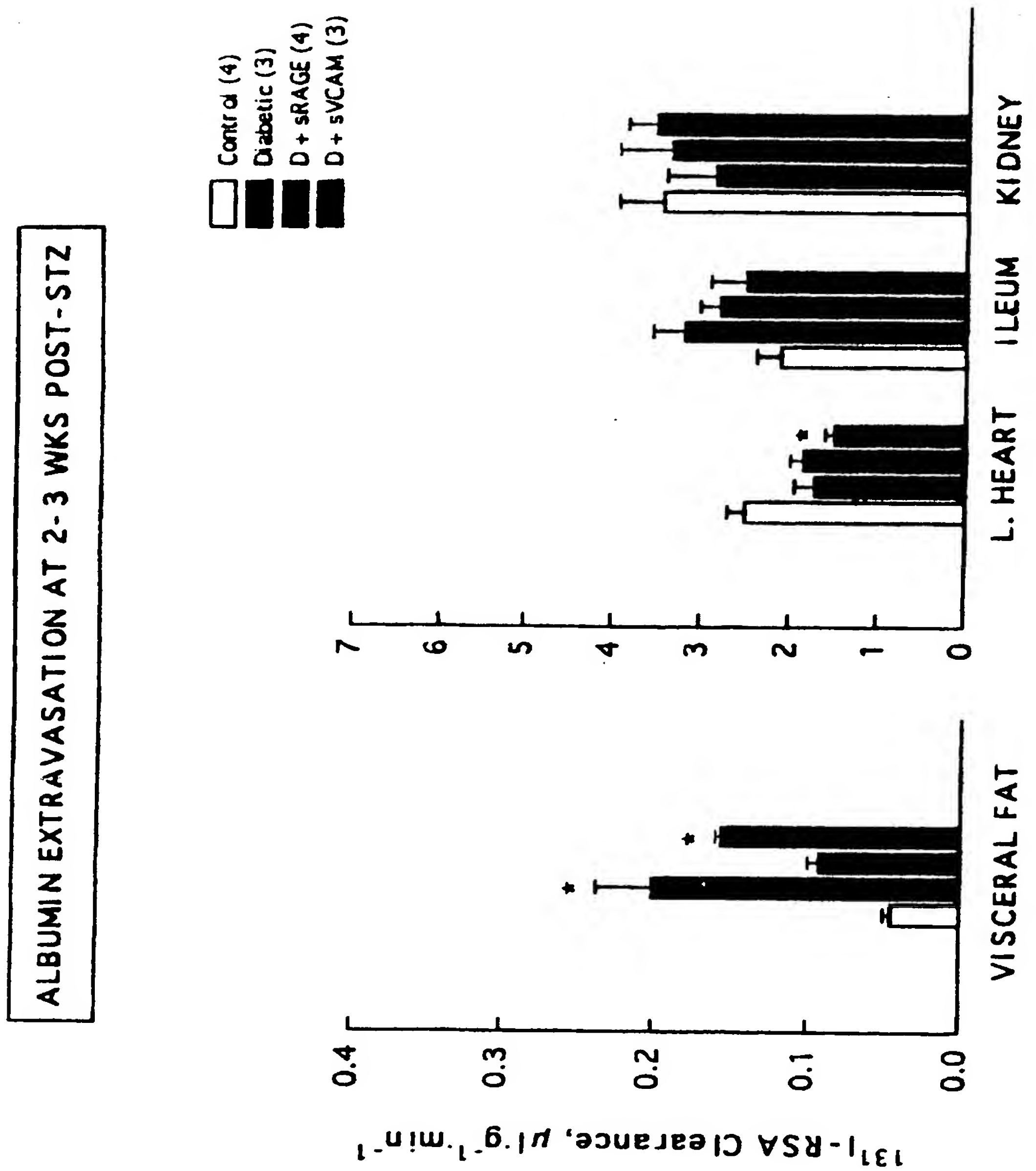


$$\text{REFERENCE VOLUME} = \frac{\text{TOTAL } ^{125}\text{I COUNTS}}{\text{PLASMA } [^{125}\text{I}]} = \text{TISSUE PLASMA VOLUME}$$

$$\text{TEST TRACER VOLUME} = \frac{\text{TOTAL } ^{131}\text{I COUNTS}}{\text{AVG PLASMA } [^{131}\text{I}]} = \text{PLASMA VOLUME + EXTRAVASCULAR DISTRIBUTION OF TEST TRACER}$$

$$\therefore \frac{\text{TEST TRACER VOLUME - PLASMA VOLUME}}{30 \text{ min}} = \text{BLOOD-TISSUE ALBUMIN CLEARANCE } (\mu\text{l/g dry wt})/\text{min}$$

Figure 12



ALBUMIN EXTRAVASATION AT 2-3 WKS POST-STZ
Control (4)
Diabetic (3)
D + sRAGE (4)
D + sVCAM (3)
^{131}I-RSA Clearance, µl.g^{-1}.min^{-1}
0.0
0.1
0.2
0.3
0.4
VISCERAL FAT
0
1
2
3
4
5
6
7
L. HEART
ILEUM
KIDNEY

Figure 13A

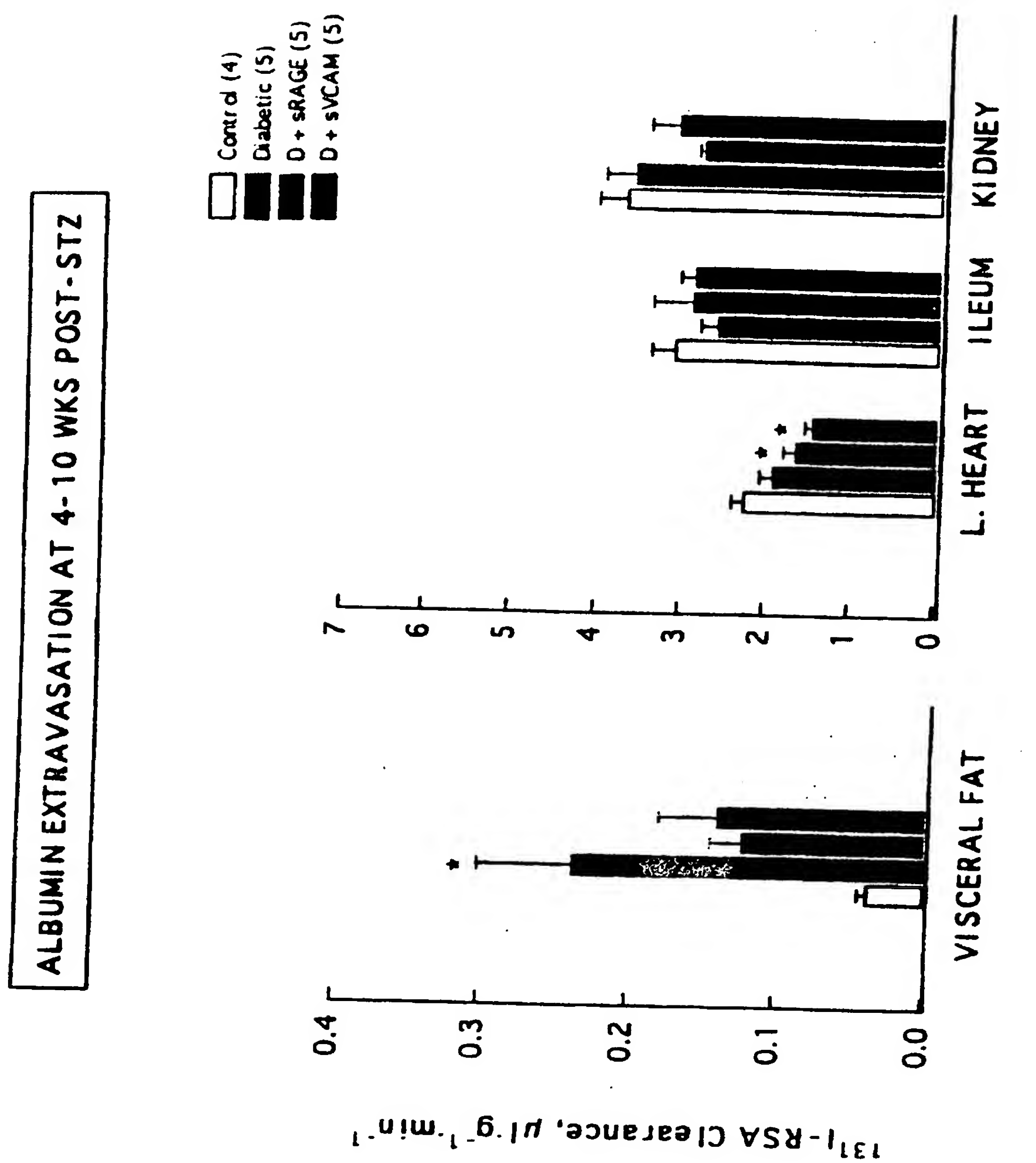

ALBUMIN EXTRAVASATION AT 4-10 WKS POST-STZ
Control (4)
Diabetic (5)
D + sRAGE (5)
D + sVCAM (5)
^{131}I-RSA Clearance, μl.g^{-1}.min^{-1}
0.0
0.1
0.2
0.3
0.4
0
1
2
3
4
5
6
7
VISCERAL FAT
L. HEART
ILEUM
KIDNEY

Figure 13B

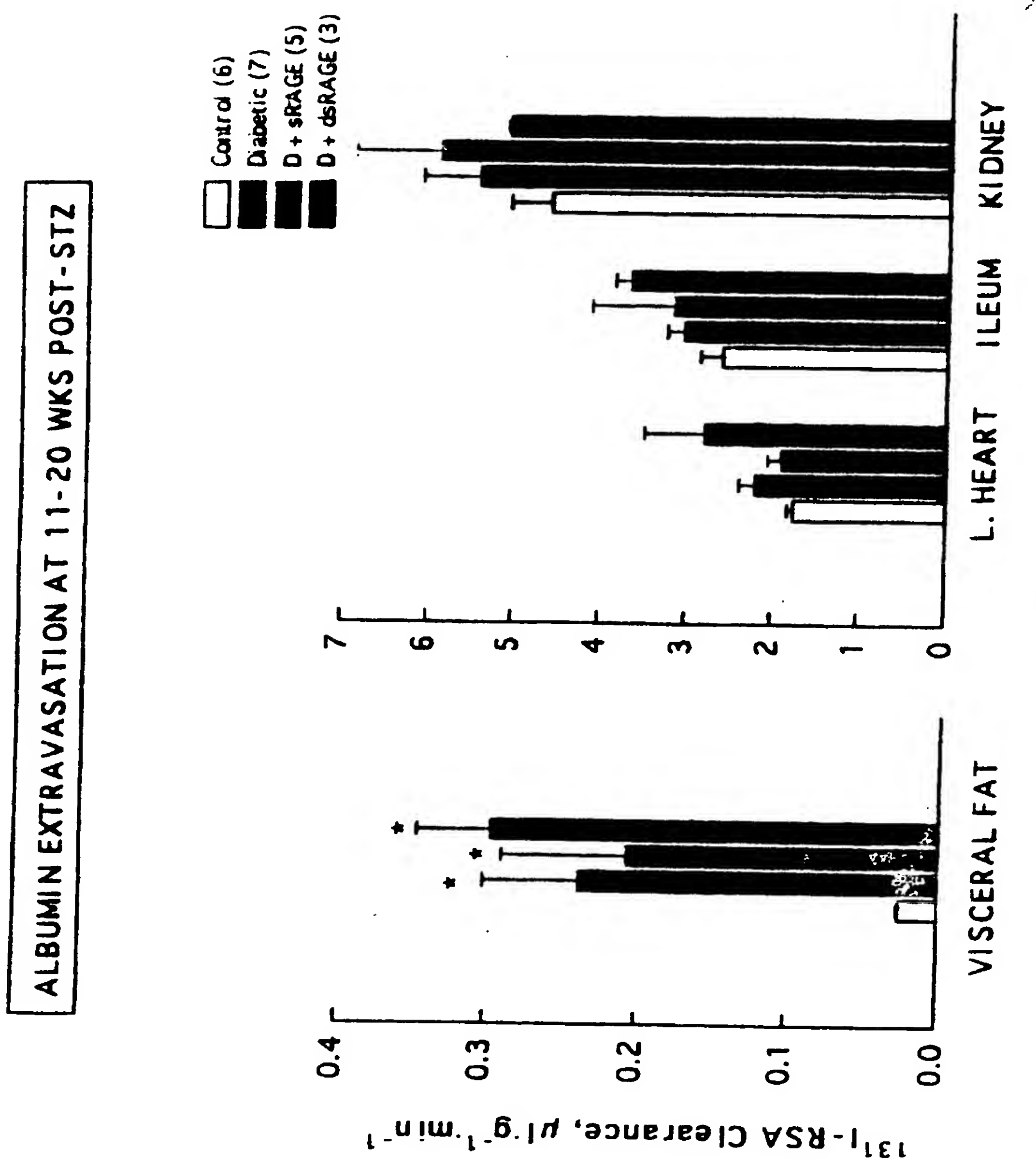

ALBUMIN EXTRAVASATION AT 11-20 WKS POST-STZ
Control (6)
Diabetic (7)
D + sRAGE (5)
D + dsRAGE (3)
^{131}I-RSA Clearance, µl.g^{-1}.min^{-1}
0.0
0.1
0.2
0.3
0.4
VISCERAL FAT
*
*
*
0
1
2
3
4
5
6
7
L. HEART
ILEUM
KIDNEY

Figure 13C

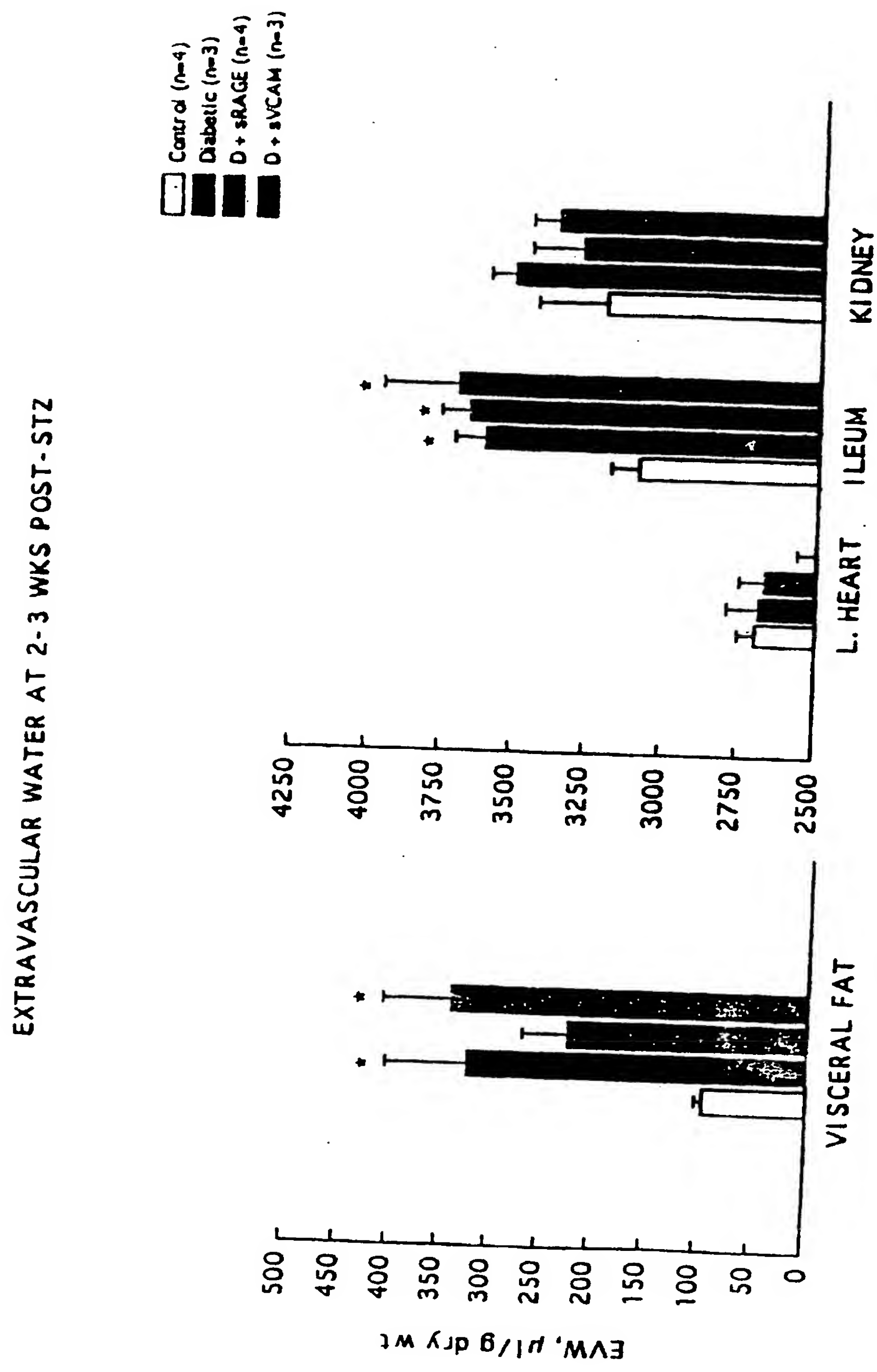

EXTRAVASCULAR WATER AT 2-3 WKS POST-STZ
Control (n=4)
Diabetic (n=3)
D + sRAGE (n=4)
D + sVCAM (n=3)
EVW, µl/g dry wt
0
50
100
150
200
250
300
350
400
450
500
2500
2750
3000
3250
3500
3750
4000
4250
VISCERAL FAT
L. HEART
ILEUM
KIDNEY
*

Figure 14A

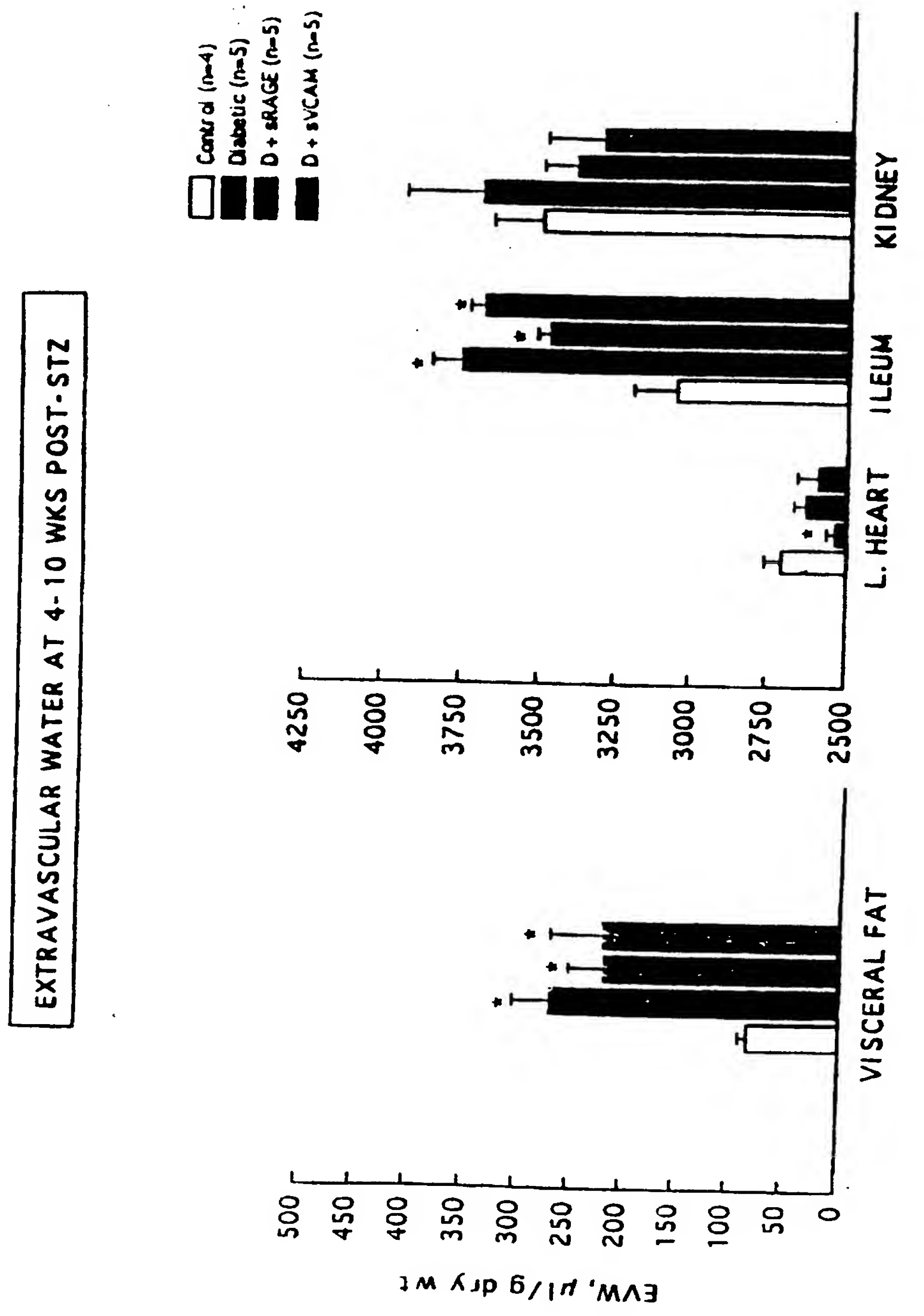

EXTRAVASCULAR WATER AT 4-10 WKS POST-STZ
Control (n=4)
Diabetic (n=5)
D + sRAGE (n=5)
D + sVCAM (n=5)
EVW, µl/g dry wt
0
50
100
150
200
250
300
350
400
450
500
2500
2750
3000
3250
3500
3750
4000
4250
VISCERAL FAT
L. HEART
ILEUM
KIDNEY

Figure 14B

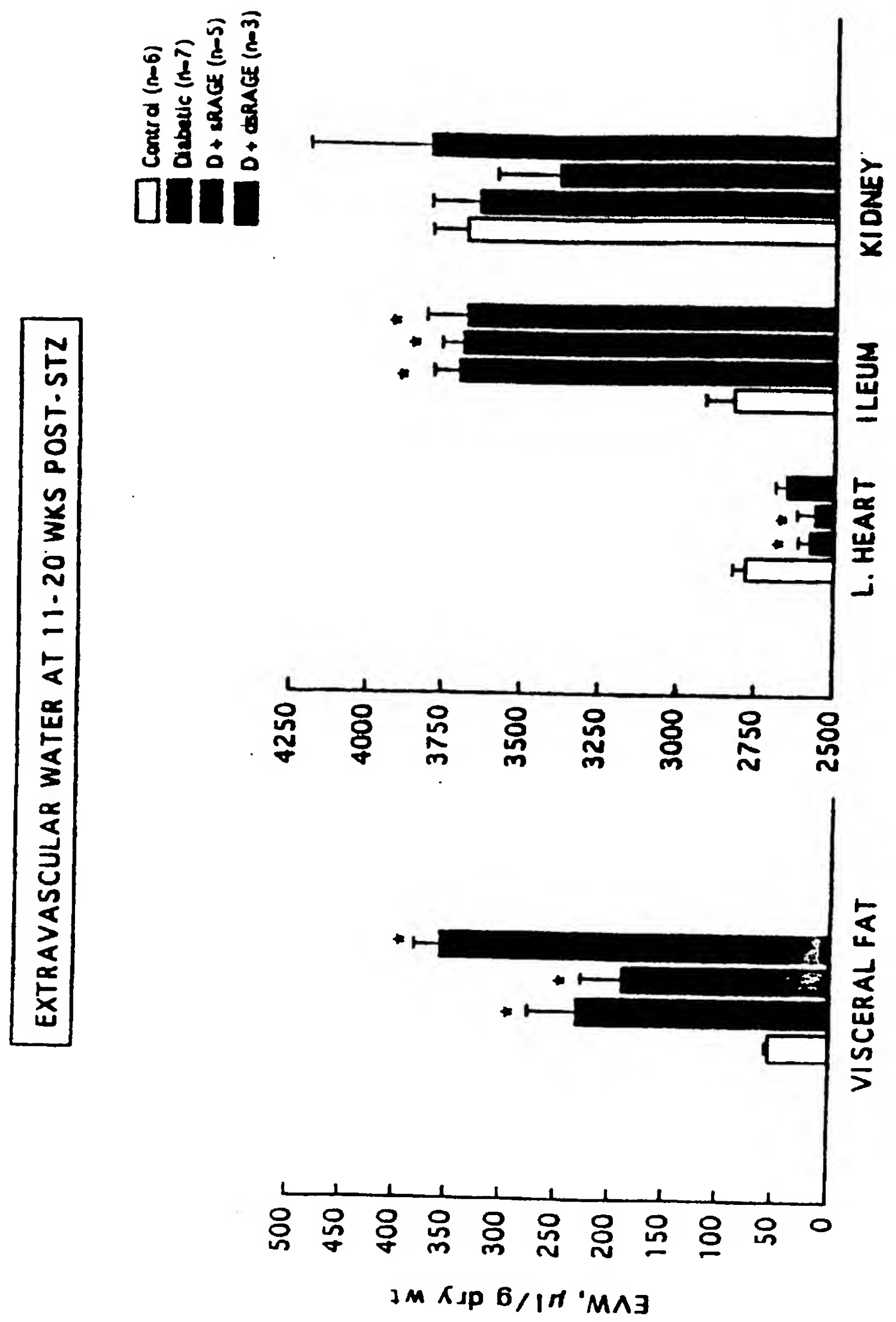
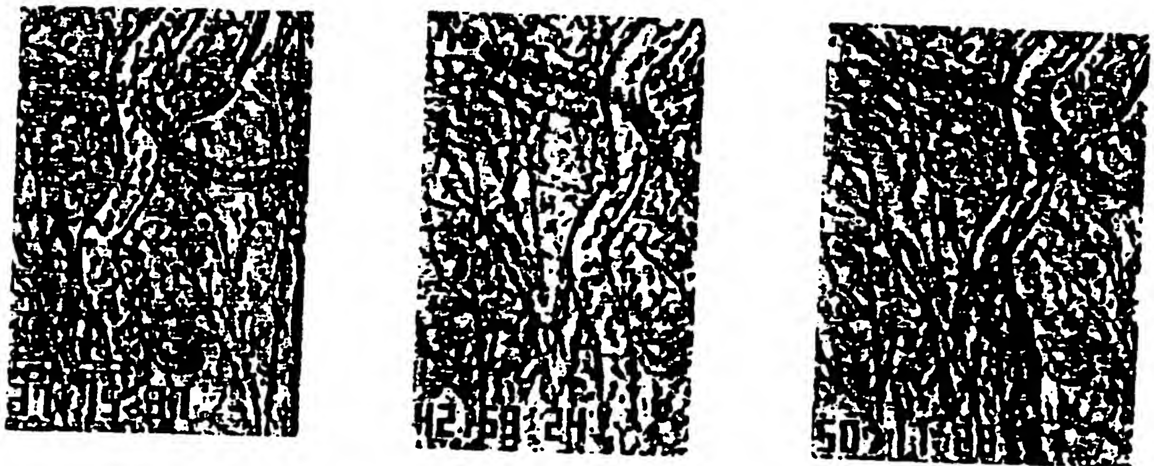
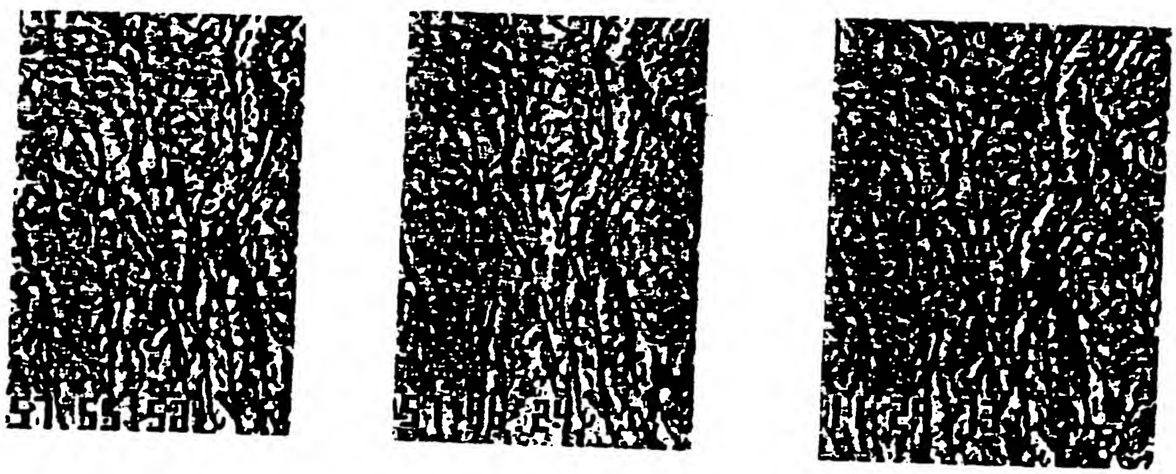

EXTRAVASCULAR WATER AT 11-20 WKS POST-STZ
Control (n=6)
Diabetic (n=7)
D + sRAGE (n=5)
D + dsRAGE (n=3)
EVW, μl/g dry wt
0
50
100
150
200
250
300
350
400
450
500
VISCERAL FAT
2500
2750
3000
3250
3500
3750
4000
4250
L. HEART
ILEUM
KIDNEY

Figure 14C

ADHERENCE OF DIABETIC RED CELLS TO VENULES IN SITU



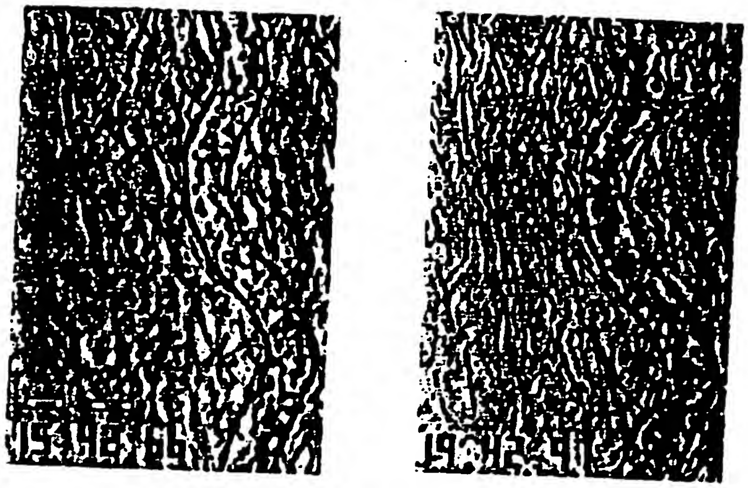
1. Control post-capillary venule perfused with diabetic RBC



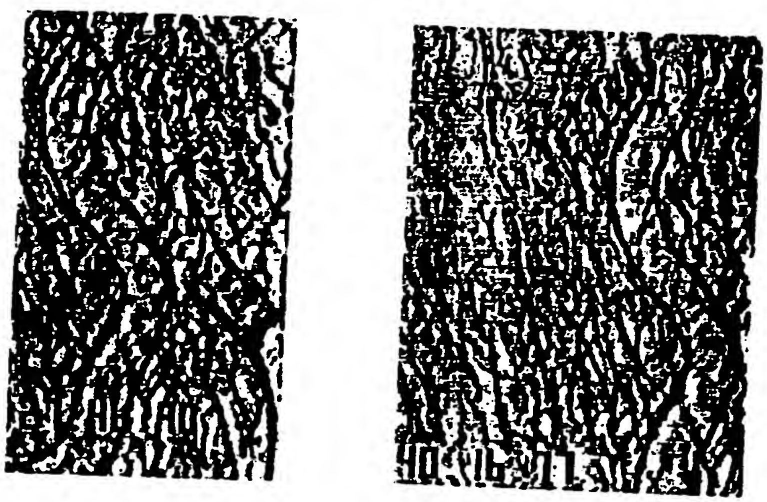
2. Same venule recannulated with Diabetic RBC + sRAGE (incubated 1 Hr)

Figure 15A

ADHERENCE OF DIABETIC RED CELLS TO VENULES IN SITU



1. Control post-capillary venule perfused with diabetic RBC + sRAGE



2. Same venule recannulated with Diabetic RBC - SRAGE

Figure 15B